THE BIOLOGY AND ECOLOGY OF THE ENTOMOPATHOGENIC NEMATODES *HETERORHABDITIS* SPP. (HETERORHABDITIDAE) AND *STEINERNEMA* SPP. (STEINERNEMATIDAE)

by

A.S. Molyneux, B. Agr. Sc. (Qld.)

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There is no material contained in this thesis which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no copy or paraphrase of material previously published or written by another person, except when due reference has been made in the text of the thesis.

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SUMMARY

The influence of a number of key environmental factors on the behaviour and survival of various species of the entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* was studied in relation to the soil habitat and the presence or absence of the insect host. In addition, the head region of adult nematodes was examined using a scanning electron microscope in order to differentiate characters of taxonomic importance because there was difficulty in separating many of the nematode strains by other means.

The association of six labial papillae with six lips was found to be characteristic of the genus *Heterorhabditis* and the paired structures of the cephalic papillae to be a potentially useful taxonomic character at the species level. The unusual lobe-like structures of the head region of an undescribed steinernematid Q1 were found to constitute a new genus within the family Steinernematidae.

In a comparison between two *Heterorhabditis* spp. (*Heterorhabditis* sp. D1 and *H. heliothidis* strain T327) and two *Steinernema* spp. (*S. feltiae* Agriotos strain and *S. glaseri* strain KG) it was found that in the absence of insect hosts, the steinernematids were able to survive for longer periods of time in sand than were the heterorhabditids. *Heterorhabditis* sp. D1, *H. heliothidis* strain T327 and *S. feltiae* Agriotos strain showed an inverse relationship between survival time and temperature whereas *S. glaseri* strain KG survived for many months at each temperature tested. Infective juveniles of *S. glaseri* strain KG became quiescent in sand when insect hosts were not available; those of the two *Heterorhabditis* spp. and *S. feltiae* Agriotos strain did not.

Heterorhabditis sp. D1 and *S. feltiae* Agriotos strain infective juveniles survived for longer periods of time in aerated water compared with infective juveniles kept in moist sand at the same temperature.

Within the pH range 4.5-6.5, the survival of *Heterorhabditis* sp. D1 infective juveniles did not appear to be affected by pH.

In a further comparison of the two genera, the steinernematids were active at lower temperatures and were able to parasitize insects over a greater temperature range. The temperature range of infectivity for insects differed between nematodes of the same genus and between species isolated from the same geographical area. However, in general, the temperature range pertaining to the nematodes' original locality tended to determine the temperature limits for nematode behaviour.

The effect of soil moisture content on the infectivity of *Heterorhabditis* sp. D1 and *S. glaseri* strain KG was investigated using post-feeding, third instar *Lucilia cuprina* larvae in various soil types. Infection occurred over a wide range of soil moisture potentials and in a loamy sand, infection occurred at moisture potentials equivalent to or below the permanent wilting point of plants (pF 4.2). In contrast, infection of *L. cuprina* larvae ceased at a lower moisture potential in a sandy clay loam and fine sand.

The infectivity of 13 species/strains of nematodes for nine species of pest insect was compared at different temperatures. All species/strains of nematodes were able to kill insects of each species. The degree of infectivity of each of the different nematodes varied considerably for different hosts and no one species/strain was the most infective for all insect species. *S. feltiae*, the only nematode species tested by most other workers, was never the most infective for any of the insect species tested and was least infective in two instances.

For the majority of soil-dwelling insects tested, the infectivity of most of the heterorhabditids was greater than that of the steinernematids. *Heterorhabditis* and *Steinernema* infective juveniles were observed entering insects via mouth, anus and/or spiracles. However, the possession of an anterior terminal tooth by *Heterorhabditis* infective juveniles enabled them to penetrate the external cuticle of insects. This additional route of entry may explain the greater infectivity of *Heterorhabditis* compared to *Steinernema* for some insect hosts.

Parasitization of an insect occurred in a temperature range that was greater than that permitting nematode development and reproduction but was less than the temperature range of growth for *Xenorhabdus* spp. The range of temperature allowing development and reproduction inside cadavers was different for each nematode species/strain tested. Different strains of the same species were also found to have different temperature limits for development and reproduction. There were also substantial differences in the number of infective juvenile nematodes produced per cadaver. Within this temperature range, cadavers resulting from exposure to high dosages of infective juvenile nematodes invariably became fetid and the nematodes failed to develop and reproduce.

Developmental rate was different for each nematode species/strain tested with most heterorhabditids taking longer to complete their life cycle than did the steinernematids at various temperatures. The relationship between developmental rate and temperature was linear. For the first time, in this area of nematology, theoretical threshold temperatures for development were calculated which gave a close approximation to the observed lower temperature limits for development.

Three particular methodologies were found to contribute to a clearer understanding and evaluation of nematode/insect bioassays:

1) The use of moist sand in specimen jars provided a uniform and easily replicated method of testing the susceptibility of soil-dwelling insects to nematode infection;

2) The relationship between dosage and mortality in the nematode/insect bioassays was described statistically by probit analysis; and

3) Post-feeding, third instar *L. cuprina* larvae were ideal for testing the influence of various environmental factors on nematode behaviour and survival in soil because:

   a) large numbers of larvae of uniform age could be easily obtained from laboratory cultures, and

   b) at this stage of their development, *L. cuprina* larvae naturally burrow into soil.

GENERAL INTRODUCTION

Nematodes of the genera *Heterorhabditis* and *Steinernema* (syn. *Neoaplectana*, Wouts *et al.* 1982) are obligate pathogens of insects in nature (Poinar 1979). These nematodes are characterized by having a non-feeding, free-living, infective-stage juvenile (dauer stage) and a mutualistic association with specific bacteria (*Xenorhabdus* spp.) (Poinar 1979; Akhurst 1980). The free-living, infective-stage juvenile carries cells of its bacterial associate monoxenically in the intestine (Thomas and Poinar 1979; Akhurst 1983a). The infective-stage juveniles are attracted to insects (Bedding and Akhurst 1975) and enter via the mouth, anus and/or spiracles (Poinar 1979). After penetrating an insect host, the nematode moves to the haemocoel where it voids its bacteria and secretes an inhibitor of the antibacterial enzymes of the insect (Götz *et al.* 1981). The bacteria proliferate, killing the insect and providing suitable conditions for growth and reproduction of the nematode (Poinar and Thomas 1967; Poinar 1979). Within about two weeks, thousands of infective juveniles are ready to infect other insect hosts (Poinar 1979).

The taxonomy of these nematodes is not well understood and only the genus *Steinernema* has been studied in any detail (Stanuszek 1972, 1974; Wouts *et al.* 1982). However, with the recent increase in the use of heterorhabditid and steinernematid nematodes as biocontrol agents of insect pests, identification of the different nematode species/strains has become more important. The ability to separate various nematode species/strains will facilitate the optimum use of these nematodes for the control of a wide range of insect hosts.

The host range of at least some of these nematodes is very wide (Poinar 1979) but most susceptibility studies have not been conducted

under field conditions. The first attempts to control insects in the field with these nematodes used *Steinernema glaseri* (syn. *Neoaplectana glaseri*) (Glaser *et al.* 1940) and initial results from test plots demonstrated nematode establishment in the soil and high insect mortality. However, in the majority of field trials, various strains of *Steinernema feltiae* (syn. *Neoaplectana carpocapsae*) were used in an attempt to control field populations of insects (Poinar 1979). Although initial results were encouraging, the failure of *S. feltiae* to control foliage-feeding insects resulted in a decline in interest in the use of these nematodes for control of insect pests. In addition, the high cost involved in the production of large numbers of nematodes restricted field testing to small areas (Gaugler 1981). However, the recent development of low cost *in vitro* mass production of *Heterorhabditis* and *Steinernema* (Bedding 1981) has now made field treatment of some insect pests over large areas economically feasible. With the successful control of some insect pests on a commercial scale (Bedding and Miller 1981a, 1981b; Miller and Bedding 1982), at a cost comparable to that of insecticides, interest in this form of biological control has been renewed.

A thorough understanding of how the infective juvenile is affected by its immediate environment is essential if these insect "pathogens" are to be successfully exploited as biocontrol agents. The soil environment is particularly important because not only is soil the natural reservoir for *Heterorhabditis* and *Steinernema* infective juveniles, it is where a large proportion of endopterygote insects spend at least part of their life cycle. The importance of soil type, moisture, and temperature on survival and behaviour of soil-inhabiting nematodes has been well

documented (Wallace 1961; Grandison 1973; Simons 1973). Reviews by Poinar (1971), Webster (1972) and Gaugler (1981) have emphasized that moisture is the most important physical factor influencing the effectiveness of entomogenous nematodes. However, most investigations on the effect of moisture (Moore 1973; Simons and Poinar 1973) and temperature (Jackson 1973; Milstead and Poinar 1978) on the survival of *Heterorhabditis* and *Steinernema* infective juveniles have not been conducted in the soil medium. The movement of these infective juveniles through soil in the presence of insect hosts has only been studied superficially and usually only with *S. feltiae* (Moyle and Kaya 1981a; Georgis and Poinar 1983). Generally, the behaviour of infective juveniles in the presence of insects has only been studied under highly artificial conditions and little effort has been made to quantify the infectivity of different nematode species for various insect hosts.

The aims of this study were to examine:

(a) the importance of cephalic and labial papillae of adult heterorhabditid and steinernematid nematodes as taxonomic characters,

(b) the influence of soil type, moisture and temperature on the survival and behaviour of the infective juvenile,

(c) the mode of entry of *Heterorhabditis* infective juveniles into different insect hosts, and

(d) the infectivity of different nematode species/strains for various insect hosts that live in or pupate in soil.

LITERATURE REVIEW

i) *Nematode Taxonomy*

Travassos (1927) established the genus *Steinernema*, incorporating *Steinernema kraussei (= Aplectana kraussei* Steiner 1923) as the type species. Steiner (1929) established the genus *Neoaplectana*, the second genus to *Steinernema* in the family Steinernematidae. Filipjev (1934a) placed *Steinernema* and *Neoaplectana* in a new sub-family, Steinernematinae, and Filipjev (1934b) noted that the *Neoaplectana* specimens were probably congeneric with *Steinernema*. However, the taxonomy of the genus *Neoaplectana* has been particularly difficult and confusing. Turco *et al.* (1971) claimed that the genus *Neoaplectana* contained thirteen different species whereas Stanuszek (1972) considered only three and Poinar (1979) recognized seven. Turco *et al.* (1971) considered that the only reliable morphological characters for taxonomy in the genus *Neoaplectana* were the spicules and gubernaculum. However, Akhurst and Bedding (1978) and Poinar (1979) noted that the gubernaculum was so variable as to be almost useless as a taxonomic character and the spicules were highly variable in some species. Using cross-breeding techniques, Stanuszek (1974) concluded that *Neoaplectana feltiae* was synonymous with *Neoaplectana carpocapsae*. Wouts *et al.* (1982) and Bedding (in press), basing their opinions on the arrangement of the cephalic papillae, labial papillae (Mráček and Weiser 1979), and the length of the male spicules and infective-stage juveniles (Mráček *et al.* 1981), now consider *Neoaplectana* to be a junior synonym of *Steinernema*. Wouts *et al.* (1982) recognized four species in the genus *Steinernema : S. kraussei, S. bibionis, S. feltiae* and *S. glaseri*.

The family Heterorhabditidae, which has a world-wide distribution (Khan *et al.* 1976; Poinar 1979; Wouts 1979; Sexton and Williams

1981; Simons 1981; Akhurst 1983a) contains only the genus *Heterorhabditis* (Poinar 1975). Only four species of *Heterorhabditis* have been described (Poinar 1975, 1979; Khan *et al.* 1976) and the taxonomy is poorly understood.

ii) *The Nematode/Bacterium Association*

Each *Heterorhabditis* and *Steinernema* species has been found associated with a single entomopathogenic bacterial species belonging to the genus *Xenorhabdus* within the family Enterobacteriaceae (Thomas and Poinar 1979; Akhurst 1982a, 1983a). The bacterium is carried monoxenically in the intestine of the infective-stage juvenile. In *Steinernema* spp. the bacteria are restricted to the ventricular portion of the intestine (Poinar and Thomas 1965; Poinar 1967) or to a vesicle in the ventricular portion of the intestine (Bovien 1937; Akhurst 1982a; Bird and Akhurst in press). In *Heterorhabditis* spp., Poinar *et al.* (1977) found bacterial cells in the ventricular portion and in the intestine proper while Wouts (1979) reported that the bacteria were restricted to the anterior portion of the intestine. The specificity of this nematode/bacterium association has been discussed by Akhurst (1983b).

The significance of the nematode/bacterium relationship has been demonstrated (Poinar 1966, 1972, 1979; Poinar and Thomas 1967; Dutky 1974; Poinar *et al.* 1980; Götz *et al.* 1981; Akhurst 1980, 1982b). The bacteria kill the insect, providing nutrients for nematode development while inhibiting the growth of other bacteria. The nematodes act as 'nematode hypodermics' (Webster 1973), transmitting the bacteria to new hosts and releasing immune inhibitors that selectively destroy the antibacterial enzymes of the insect (Götz *et al.* 1981). However, encapsulation of *S. feltiae* infective juveniles has been reported in *Aedes*

aegypti (Andreadis and Hall 1976; Poinar 1971). Axenic nematodes have also killed their insect hosts (Poinar and Thomas 1966) and Burman (1982), using axenic *S. feltiae* Agriotos strain, demonstrated the periodic production of a toxin(s) by these nematodes during their development inside the cadaver. The nematode/bacterium association has been described as being mutualistic (Poinar and Thomas 1966) or symbiotic (Bedding 1981) although Lysenko (1981) has considered the association to be of doubtful importance.

iii) *Effect of Temperature on Nematode Development and Reproduction*

Optimal temperatures for development and reproduction of many phyto-parasitic and zoo-parasitic nematodes lies in the range 20° to 30°C (Wallace 1961). A similar range for optimal nematode development and reproduction has been demonstrated for *Heterorhabdtis* spp. (Milstead and Poinar 1978; Poinar 1979; Milstead 1981) and *Steinernema* spp. (Jackson 1962; Hackett and Poinar 1973; Kaya 1977; Poinar 1979).

S. glaseri has a different temperature range from *S. feltiae* strain DD136 (Jackson 1962), however the temperature range has been shown to vary between strains of the same species. Kaya (1977), using *S. feltiae* strain DD136, demonstrated that optimal growth and reproduction in *Galleria mellonella* larvae occurred at 25°C with no growth occurring at 10°C and growth but no reproduction at 30°C. However, Hackett and Poinar (1973) reported infection and development of *S. feltiae* Agriotos strain at 34°C in adult honey bees, *Apis mellifera*.

Poinar (1979) reported variation among *Steinernema* species in their developmental rates inside a host insect at the same temperature.

iv) *Effect of Temperature on the Infective-stage Juvenile Nematode*

A number of review articles (Welch 1965; Poinar 1971; Bedding 1976; Webster 1972, 1973, 1980; Gaugler 1981) have emphasized the importance of temperature and moisture in the preparasitic environment, on the efficiency of biological control of insects by nematodes.

The short-term effects of temperature on the survival of infective-stage juveniles of *S. glaseri* (Jackson 1962) and *S. feltiae* (Schmiege 1963) have been reported. However, the infective juveniles were kept in axenic liquid media and water respectively, rather than in their natural environment. When *S. glaseri* infective juveniles were kept in water at various temperatures, their survival and reproductive potential declined with increasing age (Jackson 1973). Similar declines in survival of *S. feltiae* infective juveniles have been recorded over time under field conditions (Hoy 1954; Georgis and Hague 1981; Saunders and All 1982). Indeed, many infective stages of both zoo-parasitic and phyto-parasitic nematodes have food reserves that are rather low considering they must provide the energy needed to locate and enter the host (Croll 1970). Van Gundy *et al.* (1967), using the infective stages of *Meloidogyne javanica* and *Tylenchus semipenetrans*, directly related the depletion of food reserves with a decrease in infectivity and survival. Burman and Pye (1980a) demonstrated that the oxygen consumption and therefore utilization of food reserves of infective *S. feltiae* Agriotos strain was strongly temperature dependent and that the temperature at which they were cultured influenced oxygen uptake. Interestingly, the Q_{10} value for *S. feltiae* (Burman and Pye 1980a) in the temperature range 15° to 25°C was much higher than for the free-living nematode *Caenorhabditis elegans* (Dusenbery *et al.* 1978; Anderson 1978) and Dusenbery

et al. (1978) found that respiration of *C. elegans* was independent of growth temperature.

In common with that of almost all poikilotherms, the rate of nematode activity varies with temperature, generally increasing over the range 5° to 40°C (Croll 1970). Wallace (1963) concluded that most phyto-parasitic nematodes become inactive between 5° and 15°C; their thermal optima usually occurred between 15° and 30°C and nematode inactivity (thermal death) between 30° and 40°C. Webster (1964) using the phyto-parasite *Ditylenchus dipsaci* demonstrated that the rate of nematode activity increased linearly between 10° and 25°C. Burman (1981) noted a similar increase in activity of the infective juveniles of *S. feltiae* Agriotos strain between 12.5° and 29°C.

Croll (1967) found that the activity of *D. dipsaci* was dependent on the previous storage temperature while Burman and Pye (1980b) demonstrated active migration of *S. feltiae* Agriotos strain infective juveniles towards their growth temperatures. However, the migration of *S. feltiae* reversed direction if the infective juveniles were incubated for 6-7 days at their growth temperature while incubation of the nematodes at 2° to 5°C inhibited this reversal (Burman and Pye 1980b). Hedgecock and Russell (1975) using *C. elegans* demonstrated a similar migration towards their incubation temperature; the adult nematodes migrated towards their incubation temperature while the infective stages were repelled.

v) *Effect of Moisture on the Infective-stage Juvenile Nematode*

Laboratory studies (Dutky 1959; Schmiege 1963; Welch and Briand 1961; Jaques 1967; Kamionek *et al.* 1974) have demonstrated the high mortality of *S. feltiae* infective juveniles following rapid desicca-

tion. However, Moore (1965) assumed that *S. feltiae* infectives must be able to resist relatively low humidities in nature and Moore (1973) reported that under near saturated or saturated relative humidities, infective-stage juveniles of *S. feltiae* strain DD136 were able to move on a dry surface. Simons and Poinar (1973) supported Moore's (1965) assumption by demonstrating the ability of *S. feltiae* infective juveniles to survive gradual desiccation. Sexton and Williams (1981) recently found a natural occurrence of a *Heterorhabditis* sp. parasitizing the white-fringe weevil *Graphognathus leucoloma* in an area considered to be in drought and where irrigation had been discontinued. Survival of steinernematid infective-stage juveniles for long periods without free water in different organic solvents including paraffin oils has also been demonstrated (Bedding 1976). Furthermore, the addition of protectant materials which reduce desiccation (Webster and Bronskill 1968; Webster 1973; Bedding 1976; MacVean *et al.* 1982) has resulted in extended persistence of *S. feltiae* Agriotos strain infective juveniles when applied to foliage.

vi) *Influence of Soil Type on Infective-stage Juvenile Nematodes*

Nematode movement in soil as a function of length of nematode, pore size, moisture content and soil type for several phyto-parasitic and zoo-parasitic nematodes has been studied in detail by Wallace (1958a, 1958b, 1958c, 1959a, 1959b, 1960, 1961), Grandison (1973) and Simons (1973). Nematode movement and seasonal dynamics of *Heterorhabditis* and *Steinernema* infective juveniles in soil has only been studied superficially (Reed and Wallace 1965; Reed and Carne 1967; Danilov 1978; van Bracht [cited in Poinar 1979]; Georgis and Hague 1981; Moyle and Kaya 1981a; Mráček 1982a; Georgis and Poinar 1983). Reed and Carne (1967)

found that the infective juveniles of *S. feltiae* strain DD136 stayed near the soil surface and migrated to the soil surface when placed in the soil. In contrast, Moyle and Kaya (1981a) and Georgis and Hague (1981) observed that although the majority of infective juveniles of *S. feltiae* strain DD136 remained near the point of application, some migrated downwards. Georgis and Poinar (1983) observed similar behaviour using *S. feltiae* Breton strain in different soil types. However, the investigations were made without any regard to the importance of using comparable moisture potentials (pF values). Under field conditions, Danilov (1978) observed that *S. feltiae* Agriotos strain infective juveniles migrated randomly within the first two to four days and then entered into an inactivated state. They became active again if environmental conditions changed or in the presence of an insect host.

vii) *Orientation and Attraction of Infective-stage Juvenile Nematodes*

Although nematodes are generally considered not to orientate to pH gradients (Bird 1959), Pye and Burman (1981) showed that *S. feltiae* infective juveniles accumulated at pH values greater than 7.6. In contrast, Kuiper and deLeeuw (1963) showed that increasing soil pH beyond 7.4 had a marked nematicidal effect on some plant parasitic species. Morgan and MacLean (1968) using the nematode *Pratylenchus penetrans* found that optimum growth and reproduction in soil occurred between pH 5.5-5.8 with a rapid nematicidal effect above pH 6.6.

Bedding and Akhurst (1975) demonstrated attraction of both heterorhabditid and steinernematid infective-stage juveniles to *G. mellonella* larvae in soil. Orientation of the entomogenous nematode *S. feltiae* to carbon dioxide was demonstrated by Gaugler *et al.* (1980). The infective-stage nematodes have clearly defined amphids (Poinar and Leutenegger

1968; Poinar 1979) which are generally considered to be chemoreceptors in nematodes (Wright 1980). Several chemosensory theories of insect location by these nematodes in soil have been proposed (Schmidt and All 1978, 1979; Pye and Burman 1978, 1981; Gaugler *et al.* 1980) and recently, Byers and Poinar (1982) have postulated a thermosensory theory. The molecular basis of thermal reception has recently been discussed by Dusenbery (1980).

viii) *Insect Penetration by Infective-stage Juvenile Nematodes*

The infective stages of many insect parasitic nematodes actively penetrate the cuticle of their hosts. Penetration by several mermithids (Christie 1936; Poinar 1968; Petersen and Chapman 1970; Obiamiwe and MacDonald 1973) and tylenchids (Poinar and Doncaster 1965; Bedding 1972) has almost invariably involved the use of a stylet, often aided by glandular secretions.

The life histories of several *Heterorhabditis* spp. and *Steinernema* spp. have also been described (Poinar 1979; Wouts 1980). Early life cycle studies (Glaser 1932; Bovien 1937; Glaser *et al.* 1942; Hoy 1954) did not discuss how the infective-stage juvenile entered the insect host. Welch and Bronskill (1962) noted infection of mosquito larvae via the mouth and Weiser (1966) reported that entry through spiracles was possible. The process of infection was first studied in detail by Poinar and Himsworth (1967) using the DD136 strain of *S. feltiae* and larvae of the moth *G. mellonella*. Passive and active penetration occurred via the mouth and anus respectively, with penetration into the haemocoel through the midgut wall, apparently by mechanical pressure. Similar modes of entry by *Steinernema* infective juveniles have been described for the larvae of the large pine weevil *Hylobius*

abietis (Jackson and Moore 1969; Pye and Burman 1978) and the prepupal stage of the larch sawfly *Cephalcia lariciphila* (Georgis and Hague 1981). Entry of infective-stage juveniles into lepidopteran pupae via spiracles was suggested by Sandner and Stanuszek (1971) and demonstrated for adult lepidoptera by Triggiani and Poinar (1976). Veremchuk (1977) stated that entry by infective juveniles of *Steinernema* spp. could also occur between body segments. Entry into insects by heterorhabditid nematodes was also assumed to be via mouth, anus or spiracles (Poinar 1975; Khan *et al.* 1976) and was demonstrated by Wouts (1979) who exposed *G. mellonella* larvae to infective-stage juveniles of *Heterorhabditis heliothidis*.

ix) *Control of Insect Pests*

Results from preliminary field trials using *S. glaseri* (Glaser and Farrell 1935; Girth *et al.* 1940; Glaser *et al.* 1940; Swain 1943) and *S. feltiae* (Dutky 1959; Moore 1965; Reed and Carne 1967; Moore 1970; Harlan *et al.* 1971; Cheng and Bucher 1972; Lam and Webster 1972; Benham and Poinar 1973; Finney and Walker 1979; Burman *et al.* 1979; Kaya and Grieve 1982) together with the potential for rearing of steinernematid nematodes on a large scale using artificial media (Glaser 1940; House *et al.* 1965; Bedding 1976; Hara *et al.* 1981) promoted interest in this group of nematodes as possible biological control organisms. Several insect orders were found to be susceptible to *Steinernema* spp. (Dutky 1974) with most susceptibility studies being conducted using *S. feltiae* (Finney and Mordue 1976; Kamionek 1977; Laumond *et al.* 1979; Poinar 1979; All *et al.* 1981; Kaya and Hara 1981; Moyle and Kaya 1981b; Silverman *et al.* 1982). Later, the heterorhabditids were also considered with similar susceptibility stud-

ies being conducted (Kaya 1978; Milstead and Poinar 1978; Poinar 1979; Finney *et al.* 1982). However, most of the susceptibility studies have been done under highly artificial laboratory conditions and little effort has been made to quantify the infectivity of different nematode species for various insect hosts.

Most attempts to control field populations of insects with nematodes have used strains of *S. feltiae* against foliage-feeding insects (Finney and Walker 1977; Poinar 1979; Lindegren *et al.* 1981). These tests have yielded inconsistent results, producing nearly as many failures as successes, but poor choice of target habitat would appear to account for many of the failures (Gaugler 1981). Solar radiation (Gaugler and Boush 1978, 1979c) and desiccation (Welch and Briand 1961; Webster and Bronskill 1968; Bedding 1976) have been shown to severely reduce nematode viability on plant foliage. The use of *S. feltiae* against soil-dwelling insects has also given inconsistent results (Lewis and Raun 1978) and the high cost involved in the production of large numbers of nematodes restricted field trials to small areas (Gaugler 1981).

However, the development of low cost methods of *in vitro* mass rearing of *Heterorhabditis* and *Steinernema* species (Bedding 1981, 1983) has now made field treatment of some insect pests over large areas economically feasible. In addition, commercial control of some insects has been demonstrated; *Steinernema bibionis* (= *Neoaplectana bibionis*) has been used against the currant borer *Synanthedon tipuliformis* (Bedding and Miller 1981a; Miller and Bedding 1982) and *Heterorhabditis heliothidis* used against the black vine weevil *Otiorhynchus sulcatus* (Bedding and Miller 1981b; Simons 1981). Recently, Jackson *et al.*

(1981) and Kain *et al.* (1982) have reported favourably on the potential for control of pasture pests over large areas. The control of wood-boring insects (Lindegren *et al.* 1978, 1981; Clearwater and Wouts 1980) is another possibility.

x) *Environmental Safety*

Kaya (1977) using *S. feltiae* and its associated bacterium demonstrated that homiothermic parasitism was unlikely because growth of both the nematode and bacterium was inhibited at temperatures above 30°C. Gaugler and Boush (1979b) reported non-susceptibility of rats to intraperitoneal and *per os* inoculation of *S. feltiae* and Poinar *et al.* (1982) found that mice were not affected by subcutaneous and intracerebral inoculation of *Xenorhabdus*. Obendorf *et al.* (1983) demonstrated similar non-susceptibility of guinea pigs, mice, rats and rabbits to the entomopathogenic bacterium *Xenorhabdus nematophilus*. The failure of *X. nematophilus* to infect the laboratory animals was thought to be due to the bacterium's inability to proliferate above 34°C, however Akhurst (1983a) has demonstrated that some *Xenorhabdus* symbionts of insect pathogenic nematodes grow at temperatures above 36°C.

GENERAL MATERIALS AND METHODS

i) *Source and Culture of Nematodes*

The sources of and the methods of culturing the 19 nematode species/strains used in this study are listed in Table I. The nematodes were either reared *in vitro* monoxenic culture using the method of Bedding (1981) or reared *in vivo* using *Galleria mellonella* (Linnaeus) larvae on moist filter paper stages or 'White-traps' (Dutky *et al.* 1962).

ii) *Source and Maintenance of Insects*

The sources of insects used throughout this study are listed in Table II. Cultures of *Lucilia cuprina* (Wiedemann), *Lucilia sericata* (Meigen) and *Calliphora vicina* Robineau-Desvoidy were maintained using methods similar to that described by Mackerras (1933). *L. cuprina* adults were kept in 28.0 cm^{3*} wire gauze cages at 28°C under artificial light with a 12h light/dark regime. *L. sericata* and *C. vicina* were kept in 75.0 cm^{3**} wire gauze cages under natural light and glasshouse conditions; temperature fluctuations ranged between 10°-15°C at night and 20°-30°C during the day. *Calliphora stygia* (Fabricius) was not maintained as a laboratory culture; flies were netted and placed in small containers at 23°C whenever eggs were required.

Eggs were obtained from fly species by placing small strips of ox liver on petri dishes into the cages. *C. vicina* laid more eggs if liver was placed under a wire framed, black plastic canopy. Larvae were fed on ox liver; *L. cuprina* and *L. sericata* larvae were reared at 28°C and 70.0% R.H. while *C. vicina* and *C. stygia* larvae were reared at 23°C and 76.0% R.H.

Heliothis punctiger Wallengren was cultured at 28°C using methods similar to those of Shorey and Hale (1965). Larvae were reared on an artificial medium consisting of navy beans (340 g), wheat germ (240 g),

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* In cubic cages with sides 28.0 cm.

** In cubic cages with sides 75.0 cm.

TABLE I

Source and Culture of Nematodes

Nematode	Strain	Growth Temperature °C	Culture method *in vitro*	Culture method *in vivo*	Source
Heterorhabditis spp.	D1	28	+		Soil[a], Darwin, Northern Territory, Australia.
	Q380	28	+		Soil[a], Yepoon, Queensland, Australia.
	V16	28	+		ex *Graphognathus leucoloma*, Geelong, Victoria, Australia.
	T310	23		+	Soil[a], Sandy Bay, Tasmania, Australia.
	T280	23		+	Soil[a], Wynyard, Tasmania, Australia.
H. heliothidis	NC	28	+		W. Wouts, DSIR, New Zealand.
	NZ	23	+		W. Wouts, DSIR, New Zealand.
	T327	23	+		Soil[a], Dysart, Tasmania, Australia.
H. bacteriophora	-	23		+	W. Wouts, DSIR, New Zealand.
Steinernema spp.	Q385	23		+	Soil[a], Yepoon, Queensland, Australia.
	Q393	23		+	Soil[a], Bowenia State Forest, Queensland, Australia.
	WI	28	+		Soil[a], Dwellingup, Western Australia, Australia.
	WII	28	+		Soil[a], Dwellingup, Western Australia, Australia.
S. bibionis	T335	23	+		ex *Otiorhynchus sulcatus*, Nicholl's Rivulet, Cygnet, Tasmania, Australia.
	N60	23		+	Soil[a], (sheep campsite), Canberra, A.C.T., Australia.
S. glaseri	KG	23	+		H.K. Kaya, University of California, Davis, CA, USA.
S. feltiae	Agriotos	23	+		G.O. Poinar, University of California, Berkeley, CA, USA.
S. kraussei	-	15	+		Z. Mráček, CSAV, Budejovice, Czechoslovakia.
Undescribed steinernematid	Q1	28	+		Soil[a], Mirani, Queensland, Australia.

[a] Isolated by the method of Bedding and Akhurst (1975).

TABLE II

Source of Insects

Insect	Strain	Source
Lucilia cuprina	AM-3	Division of Entomology, CSIRO, Canberra, Australia.
Lucilia sericata	Perth	Division of Entomology, CSIRO, Canberra, Australia.
Calliphora vicina	Stowell	Battery Point, Hobart, Tasmania, Australia.
Calliphora stygia	-	Battery Point, Hobart, Tasmania, Australia.
Galleria mellonella	-	Division of Entomology, CSIRO, Hobart, Tasmania, Australia.
Heliothis punctiger	Indooroopilly	R.E. Teakle, Department of Primary Industry, Queensland, Australia.
Adoryphorus couloni	-	Ross district, Tasmania, Australia.
Otiorhynchus sulcatus	-	Pyengana, Tasmania, Australia.
Sitona humeralis	-	P.T. Bailey, Sth Aust. Dept. of Agri., South Australia, Australia.

yeast (200 g), agar (50 g), ascorbic acid (12 g), methyl para-hydroxy benzoate (Nipagin M) (12 g), sorbic acid (4 g), 40.0% formaldehyde (4 ml) and tap water (3.1 *l*). *G. mellonella* larvae were cultured at 32°C on an artificial medium of Farex (Glasco, Australia) (50 g), glycerine (22 g), honey (23 g) and yeast (5 g), (Dutky *et al.* 1962).

Adoryphorus couloni (Burmeister), *Otiorhynchus sulcatus* (Fabricius) and *Sitona humeralis* Stephens were collected from the field and kept at 15°C in moist peat with sliced carrot for four days (to allow for collection damage to result in mortality) before being used in experiments.

iii) *Infection of Insects by Infective-stage Juvenile Nematodes*

Insect larvae were exposed individually to nematode infection within plastic screwcap specimen jars (diameter 4.2 cm, height 6.0 cm) filled to within 1.0 cm of the top with approximately 80 g of clean fine sand, moisture content 7% (pF = 1.3). Infective-stage nematodes were introduced in 1.0 ml of water into a centrally placed well (0.5 cm diameter, 2.0 cm deep), which was then filled with sand and left for about 16h to allow equilibration at the required temperature. Dosages of one to ten nematodes were individually counted whereas dosages greater than ten were estimated by dilution counts. One insect larva was placed on the sand surface and the lid screwed on after ensuring that the larva had started to burrow. *G. mellonella* larvae, which pupate above ground, were placed in the containers before the sand and nematodes were added. After 10-14 days at temperatures of 18°C or more and 28 days at temperatures below 18°C, the jars were emptied and the sand sieved in water. The larvae or pupae found were microscopically examined in insect Ringer's solution (Cruickshank *et al.* 1970) for nematode parasitization.

SECTION I

A COMPARISON BETWEEN THE HEAD END SENSILLA OF ADULT *HETERORHABDITIS* AND *STEINERNEMA* NEMATODES

INTRODUCTION

Steiner (1923) established *Aplectana kraussei* a newly found nematode parasite of the sawfly *Cephalcia abietis* as a new species in an already existing genus but Travassos (1927) made *A. kraussei* the type species of the new genus *Steinernema*. When Steiner (1929) established the genus *Neoaplectana*, this became the second genus to *Steinernema* in the family Steinernematidae. The two genera were distinguished by the number and arrangement of the head sense organs and by the number of male copulatory papillae. Unfortunately, Steiner did not describe completely the tail papillae of *S. kraussei*. However, Mráček (1977), in the redescription of *S. kraussei*, found that the number of male copulatory papillae was the same as reported by other workers (Bovien 1937; Poinar 1967) for the genus *Neoaplectana*. Therefore, the only character that separated the two genera was the number and arrangement of the head sense organs.

Steiner (1929) described *Neoaplectana* as having an outer circle of six cephalic papillae and an inner circle of six labial papillae. In comparison, *S. kraussei* (Steiner 1923) was described as having four cephalic (sub-medial) papillae and labial papillae were not mentioned.

Although Filipjev (1934b) had previously noted that specimens of *Neoaplectana feltiae* were probably congeneric with *Steinernema*, the original descriptions of the head sense organs of both genera were considered correct. Steiner's authority remained unchallenged until Mráček *et al.* (1981) and Bedding (in press) suggested that the two genera may be congeneric. Recent scanning electron micrographs have shown that the number and arrangement of cephalic and labial papillae of *Neoaplectana* and *Steinernema* are identical and the two genera are now

considered congeneric, with *Steinernema* having precedence (Wouts *et al.* 1982).

The taxonomy of the recently described genus *Heterorhabditis* (Poinar 1975) is poorly understood. There is only one genus within the family Heterorhabditidae and although the heterorhabditids are clearly distinguished from the steinernematids (Poinar 1979), the apparent lack of suitable morphological characters has made separating the species of *Heterorhabditis* particularly difficult. As yet, no scanning electron microscopical studies of the head sense organs of *Heterorhabditis* spp. have been reported.

In this section, the scanning electron microscope (S.E.M.) was used to examine the head sense organs of several undescribed *Heterorhabditis* spp. and *Steinernema* spp. in an attempt to find morphological characters that may be useful in their taxonomic classification.

MATERIALS AND METHODS

i) *Specimen Preparation*

First generation adult heterorhabditid and steinernematid nematodes were dissected in Ringer's solution from *G. mellonella* larvae that had been infected in sand and left at 23°C for five and three days respectively. Adult nematodes were washed several times in insect Ringer's solution followed by a brief wash in distilled water. They were then transferred using a mounted hair and fixed by immersion in a mixture of 5% glutaraldehyde and 4% paraformaldehyde in 0.1 M Na-cacodylate buffer at pH 7.3 for 2h at 23°C. Specimens were then washed in several changes of buffer and post-fixed for 1h at 23°C in 1% osmium tetroxide in the same buffer. Specimens were again washed in several changes of buffer

followed by a brief wash in distilled water. Prior to dehydration, adult nematodes were cut in half in order to facilitate penetration of ethanol. Specimens were dehydrated for 1h in 30% and 50% ethanol, 16h in 70% ethanol, 1h in 80%, 90% and 95% ethanol and 3 x 45 min in fresh absolute alcohol.

ii) *Examination of Specimens*

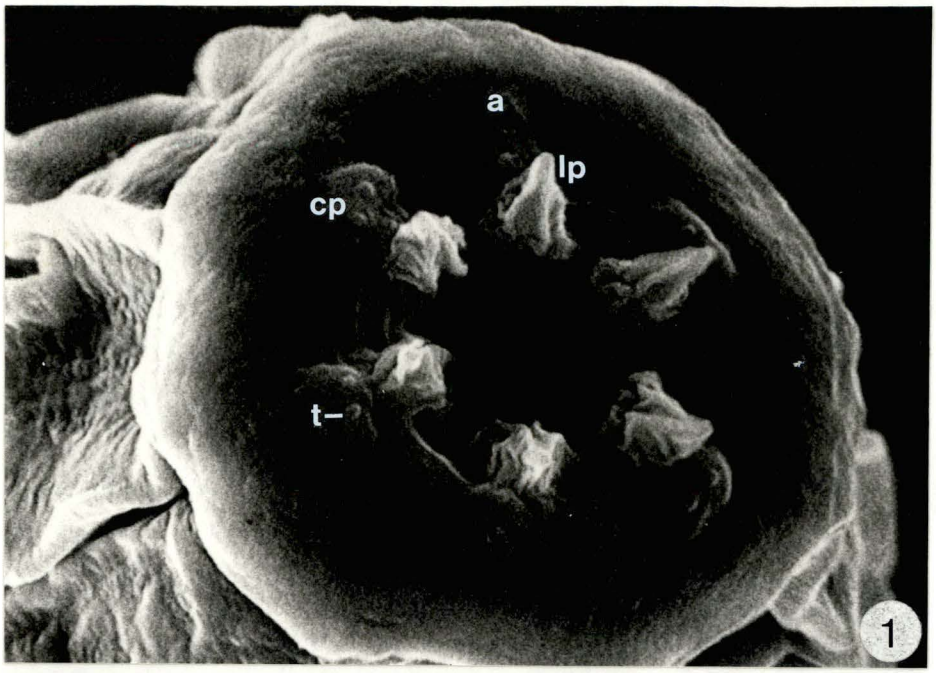
Adult nematode preparations were air dried and mounted onto brass S.E.M. (1.0 cm diameter) stubs using double sided adhesive tape (Scotch, Australia Pty. Ltd.). Silver conductive paint was applied to ensure contact between specimen and upper surface of stub. Specimens were coated with gold/pallidium at 300Å in a Jeol Sputter JFC-1100 and examined with a Jeol JSM-35 scanning electron microscope.

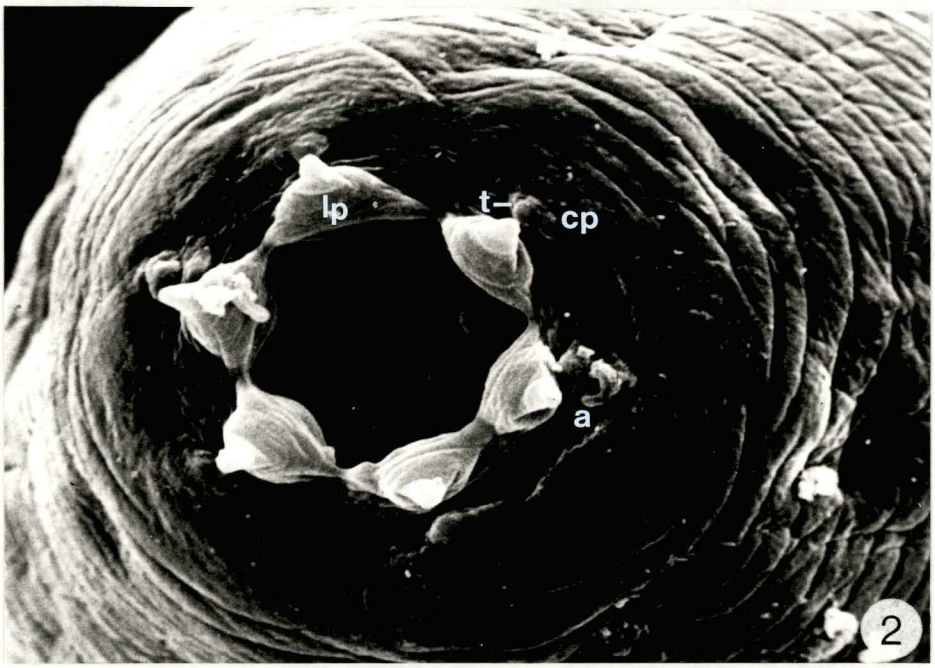
OBSERVATIONS AND RESULTS

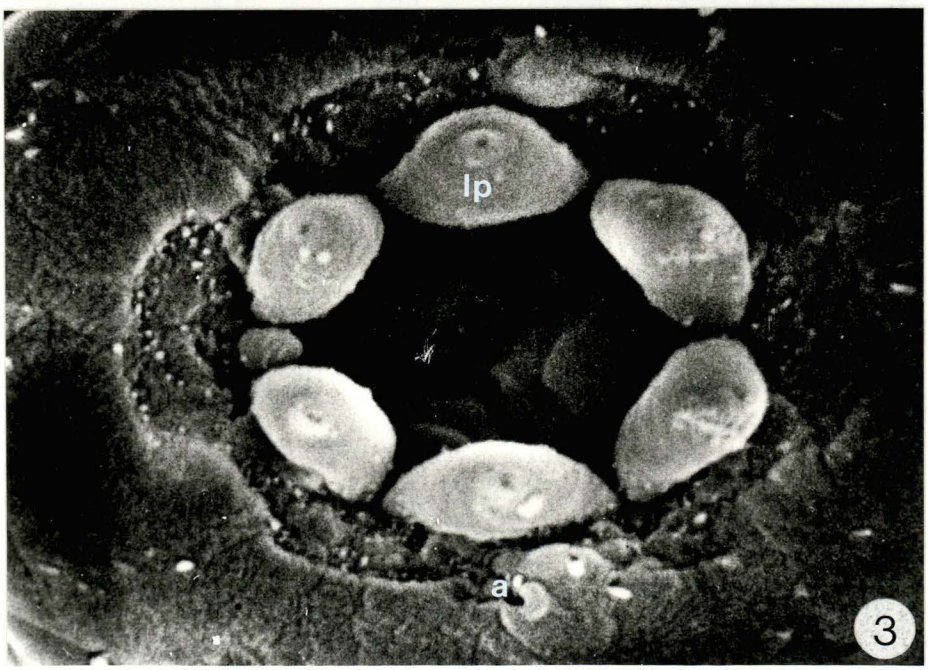
Examination of first generation female *Heterorhabditis* sp. D1 (Fig. 1), *H. heliothidis* strain T327 (Fig. 2) and *H. heliothidis* strain NZ (Fig. 3) showed that each nematode had six protruding lips surrounding the mouth opening. Each lip had a labial papilla at the distal end (Figs. 1-3) and this arrangement was consistent for all *Heterorhabditis* spp. examined (e.g. *Heterorhabditis* spp. V16, Q380, T310, T280; *H. heliothidis* NC strain and *H. bacteriophora*). There were also four cephalic papillae forming an outer circle behind the six lips, except in *H. heliothidis* NZ strain (Fig. 3) where, although the amphids could be seen clearly, there was no suggestion of any cephalic papillae. When the cephalic papillae were present, each papilla was found to have two distinct tuberculi giving the papilla an appearance of being a paired structure (Figs. 1 and 2).

Figs. 1-3 Scanning electron micrographs (S.E.M.) of *En-face* view showing protruding lips and papillae of first generation adult female *Heterorhabditis* spp. 1: (4,000x) S.E.M. of *Heterorhabditis* sp. D1. 2: (3,300x) S.E.M. of *Heterorhabditis heliothidis* strain T327. 3: (4,800x) S.E.M. of *Heterorhabditis heliothidis* strain NZ.

Key: a, amphid; cp, cephalic papillae; lp, labial papillae; t, tuberculi.


a
lp
cp
t–
1


lp
t–
cp
a
2


lp
a
3

Figs. 4-6 Scanning electron micrographs (S.E.M.) of *En-face* view showing head papillae of first generation adult female *Steinernema* spp. and an undescribed steinernematid. 4: (3,400x) S.E.M. of *Steinernema glaseri* strain KG. 5: (2,600x) S.E.M. of *Steinernema feltiae* Agriotos strain. 6: (3,000x) S.E.M. of undescribed steinernematid Q1.

Key: a, amphid; cp, cephalic papillae; lp, labial papillae.


lp
cp
4


cp
ip
5


a
cp
lp
6

In comparison, scanning electron micrographs of *S. glaseri* KG strain and *S. feltiae* Agriotos strain (Figs. 4 and 5) showed an absence of any lips associated with the oral cavity. Instead, the mouth opening was surrounded by an inner circle of six labial papillae and an outer circle of four unpaired cephalic papillae each having only one tubercule (Figs. 4 and 5). These results were consistent for all *Steinernema* spp. examined (e.g. *Steinernema* spp. Q385, Q393, WI, WII; *S. bibionis* T335 strain and *S. kraussei*).

Examination of the undescribed steinernematid Q1 nematode demonstrated a similar pattern of six labial papillae surrounded by four unpaired cephalic papillae (Fig. 6). However, the overall structure of the oral cavity did not resemble either the *Heterorhabditis* or *Steinernema* nematodes. Instead, the labial papillae were located at the distal end of six large protuberances which actually formed at least part of the oral cavity. This very different characteristic together with other morphological differences (Bedding, unpublished data) clearly separates the nematode Q1 from *Heterorhabditis* and *Steinernema* nematodes and indicates that it warrants generic status.

DISCUSSION

The basic arrangement of head papillae of rhabditoid nematodes includes 16 papillae in three concentric circles around the oral opening, 12 being labial (6 + 6) associated with six lips, and four cephalic (De Coninck 1965). The scanning electron micrographs of adult heterorhabditid and steinernematid nematodes revealed that the labial and cephalic papillae are in two circles; the inner labial papillae were hexagonally distributed and the outer cephalic papillae had a tetragonal

distribution. There are only six labial papillae in these nematodes but whether this is because of a reduction of the inner or outer labial ring existing in free-living nematodes is not known (Mráček and Weiser 1979). In the genus *Heterorhabditis*, the six labial papillae are also associated with corresponding lips (Figs. 1-3) whereas the lips were reduced or completely absent in the genus *Steinernema*. This suggests that the heterorhabditid arrangement has a closer affinity with the basic rhabditoid structure.

The overall results indicated that the number of labial and cephalic papillae are not important taxonomic characters for the separation of the genera *Heterorhabditis* and *Steinernema* or for separation of different *Steinernema* species. However, the paired structure of the cephalic papillae (Figs. 1 and 2) and/or the location of labial papillae at the distal ends of lips (Figs. 1-3) characteristic of the genus *Heterorhabditis*, appear to be potentially useful taxonomic characters at the species level. Furthermore, the absence of cephalic papillae (Fig. 3) separates *H. heliothidis* strain NZ from *H. heliothidis* strain T327 (Fig. 2) and from the original type culture of *H. heliothidis* strain NC and suggests that *H. heliothidis* NZ is a different *Heterorhabditis* species.

The undescribed steinernematid Q1 (Fig. 6) more closely resembles the genus *Steinernema* in number and arrangement of head papillae. However, although the location of labial papillae at the distal ends of large protuberances might suggest that the phylogeny of the Q1 nematode lies in between the Steinernematidae and the Heterorhabditidae, other morphological characters closely resemble *Steinernema* spp. but are very different from *Heterorhabditis* (Bedding pers. comm.). Nevertheless, the differences of the cephalic region of Q1 do warrant the erection of a new genus for this species (Bedding pers. comm.).

SECTION II

THE INFLUENCE OF SOIL TYPE, MOISTURE, TEMPERATURE AND pH ON THE SURVIVAL AND BEHAVIOUR OF INFECTIVE-STAGE JUVENILE NEMATODES IN THE SOIL ENVIRONMENT

INTRODUCTION

The non-feeding, soil-dwelling *Heterorhabditis* and *Steinernema* infective juveniles are attracted to insects in soil (Bedding and Akhurst 1975) and enter via the insect's natural openings (Poinar 1979). Although promising results were obtained using *S. glaseri* (Glaser *et al.* 1940; Swain 1943) and *S. feltiae* (Jaques *et al.* 1968; Harlan *et al.* 1971; Benham and Poinar 1973) against soil-inhabiting insects in the field, most field trials have involved foliar applications. However, with the failure of *S. feltiae* to control foliage-feeding insects, greater emphasis has again been placed on the treatment of soil-inhabiting insects (Gaugler 1981; Bedding and Miller 1981b). Advances with *in vitro* nematode culture (Bedding 1981, 1983) have now made possible large-scale testing of these nematodes against soil-inhabiting insects, and the favourable reports of Jackson *et al.* (1981) and Kain *et al.* (1982) have emphasized the need to study environmental factors that influence the survival and behaviour of these nematodes in soil.

Three of the most important environmental factors influencing soil-dwelling nematodes are soil type, moisture and temperature (Wallace 1961; Simons 1973). Reviews by Poinar (1971) and Webster (1972) have emphasized that moisture is the most important physical factor influencing the effectiveness of entomogenous nematodes. The effect of moisture on survival of steinernematid nematodes has only been studied superficially (Welch and Briand 1961; Kamionek and Sandner 1974; Simons and Poinar 1973) and only with *S. feltiae* in relation to survival on foliage or artificial surfaces. The effect of moisture on these nematodes in soil has not been studied, although the variability of results from

field trials has often been attributed to insufficient soil moisture (Glaser *et al.* 1940; Lewis and Raun 1978; Kain *et al.* 1981).

The relationship between soil moisture, moisture potential (pF values) and soil type and their effect on movement of soil-inhabiting nematodes has also been investigated (Wallace 1960). Although the effects of soil moisture on the movement of *S. feltiae* on the soil surface have been described (Reed and Wallace 1965), studies on the movement of *S. feltiae* in soil (Reed and Carne 1967; Moyle and Kaya 1981a) have not considered the effects of soil moisture. Although Georgis and Poinar (1983) studied the influence of soil texture on the movement of *S. feltiae* in different soil types, they apparently did so without regard to the importance of comparable pF values.

The effect of temperature on the survival and behaviour of the infective juvenile nematode has only been studied superficially using *S. glaseri* (Jackson 1962, 1973) and *S. feltiae* (Schmiege 1963). Moreover, the nematodes were kept in axenic liquid media and water respectively, rather than in their natural soil environment. Optimal temperatures for development and reproduction of some *Heterorhabditis* and *Steinernema* species have been found to lie between 20° and 30°C (Poinar 1979) although development and reproduction over a wide temperature range has only been determined for *S. feltiae* strain DD136 (Kaya 1977).

The effect of pH on the survival of *Heterorhabditis* and *Steinernema* infective juveniles has not been reported, although Pye and Burman (1981) showed that *S. feltiae* accumulated at pH values greater than 7.6.

This section investigates the influence of soil type, moisture temperature and pH on the survival and behaviour of infective juveniles of a number of nematode species/strains in the presence and absence of

insect hosts. Furthermore, the effect of temperature on nematode development and reproduction inside cadavers is studied using *L. cuprina* and *G. mellonella* larvae.

MATERIALS AND METHODS

i) *Low Temperature Immobilization of Infective Juvenile Nematodes*

Infective-stage juveniles of the 16 nematode species/strains were initially obtained from cultures maintained by the CSIRO Division of Entomology, Hobart (Table 1) and reared *in vivo* using *G. mellonella* larvae at 23°C prior to these experiments.

Clean active suspensions of infective juveniles were obtained by allowing the nematodes to migrate through a modified Baermann sieve (diameter 16.0 cm, depth 1.0 cm) overlaid with two sheets of tissue paper, at 23°C (Whitehead and Hemming 1965). Nematode suspensions were diluted to 10^3 infective juveniles per ml and 0.1 ml aliquots were transferred on 1.0 cm^2 pieces of filter paper to 1.0% purified agar in petri dishes. The petri dishes were then incubated at the required temperatures. Temperatures equal to or greater than 4°C were obtained using controlled temperature rooms or incubators. For temperatures below 4°C, more precise measurements were made using a temperature gradient incubator, (Model TN-3 Toyo, Ragaku, Sangyo, Co., Ltd., Tokyo, Japan) which was housed in a controlled temperature room at 10°C. A temperature gradient from -3° to 4°C was obtained at intervals of 0.3°C per cm.

The activity of nematodes was assessed microscopically. Nematodes were classed as inactive if no movement occurred after prodding with a mounted hair, as barely active if they moved part of their body and active if able to move along the agar surface when touched.

ii) *Effect of Temperature on the Parasitization of Insect Hosts by Infective Juvenile Nematodes*

Infective juveniles were obtained from *in vivo* cultures at 23°C to ensure a uniform age of infective nematodes of between one and seven days.

Parasitization of post-feeding third instar *L. cuprina* larvae by *Heterorhabditis* and *Steinernema* infective juveniles was studied at various temperatures in sand. A dosage of 320 infective juveniles (found to be more suitable by preliminary investigation) was used for each nematode species/strain with 20 replications for each nematode/temperature combination. It was not feasible to examine simultaneously the full spectrum of nematodes. The 16 nematode species/strains were divided into four groups; when each group was tested *Heterorhabditis* sp. D1 was included as a standard and there were 20 nematode-free controls.

Nematode development and reproduction was also recorded for each temperature.

iii) *Quantitative Analysis of Insect Susceptibility at Different Temperatures*

The susceptibility of *L. cuprina* larvae to 12 nematode species/strains was analyzed quantitatively for three temperatures, 18°, 23° and 28°C. For each nematode/temperature combination there were eight dosages of infective juveniles : 10^0, 10^1, $10^{1.5}$, 10^2, $10^{2.5}$, 10^3, $10^{3.5}$, 10^4. Each combination of nematode/temperature/dosage was tested on two occasions, with each combination consisting of 20 specimen jars. In addition to the duplicate tests of each treatment combination there were also some additional tests for some dosages prepared for

previous preliminary experiments. These extra tests had been conducted at all temperatures. It was not feasible to examine simultaneously the full spectrum of nematodes; each experiment consisted of three nematode species/strains, 20 nematode-free controls and *Heterorhabditis* sp. D1 as a standard.

The probit analysis of Finney (1971) was performed using the generalised linear model facility of the GENSTAT statistical package (Alvey *et al.* 1977). Probit lines were fitted as regressions between percent parasitized and $\log_{10}$ dosage using the probit link and binomial error specifications of GENSTAT. The analyses were carried out in two stages. Firstly, the coincidence of probit lines within the duplicate pairs was tested using the deviance measure computed by GENSTAT. For a given fitted model the deviance is a measure of the discrepancy between the data and the corresponding fitted values derived from the model. The change in deviance between fitting individual lines to each duplicate and fitting a common line to each pair of duplicates is distributed approximately as chi square (χ^2) with the appropriate degrees of freedom. A significant change in deviance implies that the null hypothesis of coincident lines should be rejected. The null hypothesis was not rejected in this case and LD_{50} and LD_{90} values were calculated for each nematode/temperature combination based on the probit lines common to both duplicates. The second stage involved selected comparisons between nematode/temperature combinations. Comparisons were made as pairwise tests of coincident lines between the selected nematode/temperature combinations in a similar manner to the first stage.

iv) *Pupariation Times of L. cuprina Larvae for Different Temperatures*

Sand-filled specimen jars were left overnight at the required temperatures and *L. cuprina* larvae added the following day. Post-feeding third instar *L. cuprina* larvae were individually placed into specimen jars filled with sand and kept at 12°, 15°, 18°, 23°, 28° and 35°C. For each temperature, 50 replications were washed out every 12h except for 15° and 12°C where 50 replications were washed out every seven days. The number of larvae and puparia were recorded. The prepuparial stage was considered finished when the puparia were rounded at both ends and could no longer extend themselves when touched (Fraenkel and Bhaskaran 1973).

v) *The Effect of Temperature and pH on the Behaviour and Survival of Infective Juvenile Nematodes*

a) *In Soil - Heterorhabditis* sp. D1, *H. heliothidis* strain T327, *S. glaseri* strain KG and *S. feltiae* Agriotos strain infective juveniles were kept in sand-filled specimen jars at 10°, 15°, 23° and 28°C for 2^0 to 2^5 weeks. The moisture content of the sand was kept at approximately 7% (pF=1.3) by enclosing the specimen jars with water saturated tissue paper in heavy duty plastic. A constant dosage of 10^3 infective juveniles of uniform age (one to seven days) was introduced in 1.0 ml of water into the sand for each nematode species/strain. There were 60 replications for each nematode/temperature/time combination.

At the required intervals, 56 replications of each nematode/temperature combination were placed at 23°C and left for about 16h to allow equilibration at that temperature. A single *L. cuprina* larva was then placed on the sand surface of each of 50 jars, the

lids screwed on and the jars left at 23°C. Final instar *G. mellonella* larvae were individually placed into the remaining jars and left at 23°C. After 10 days, the jars were emptied and the sand sieved in water. The *L. cuprina* larvae and/or pupae found were dissected in insect Ringer's solution and microscopically examined for nematode parasitization while the *G. mellonella* larvae were microscopically examined for nematode development and reproduction. If there was no parasitization of *L. cuprina* larvae, then final instar *G. mellonella* larvae were used to measure nematode infectivity at the later sampling times.

At the same time, nematode survival in sand was measured by emptying the sand from specimen jars onto modified Baermann sieves at 23°C. There were four replications for each nematode/temperature/time combination. At the end of each 24h interval the water together with nematodes was poured into 250 ml beakers and after sedimentation of the nematodes and decanting of excess water the remaining nematodes were microscopically counted at 12x magnification.

The survival of *Heterorhabditis* sp. D1 infective juveniles in sand was also compared with their survival in loamy sand, peat, vermiculite and heat sterilised sand at comparable moisture contents.

b) *In Aerated Water* - *Heterorhabditis* sp. D1 and *S. feltiae* Agriotos strain infective juveniles were aerated in water at a density of 8,000 nematodes per ml at 23°C for 18 weeks. At two weekly intervals nematode mortality was determined and the nematodes infectivity for *L. cuprina* larvae in sand-filled specimen jars at 23°C was tested. Dosages of 10^1, $10^{1.5}$, 10^2, $10^{2.5}$ and 10^3 infective juven-

iles were used and there were 20 replications for each nematode/dosage/time combination plus 20 nematode-free controls.

The generalised linear regression models in GENSTAT (Alvey *et al.* 1977) were used to test for differences in the infectivity of *Heterorhabditis* sp. D1 and *S. feltiae* infective juveniles for *L. cuprina* over time.

c) *Effect of pH on the Survival of Heterorhabditis sp. D1 Infective Juveniles* - Buffer solutions of different pH values ranging from 4.5 to 6.5 were established using mixtures of 0.1 M-citric acid and 0.2 M-Na_2HPO_4 (McIlvaine 1921). Buffered agar solutions were also made using purified agar (Bacto). Either 5.0 ml of buffered agar or 2.0 ml of buffered solution were added to each 10.0 ml McCartney bottle and sterilized by autoclaving. Bottles containing agar were rotated under cold water to spread an even film of agar over the walls and base.

One to seven day old *Heterorhabditis* sp. D1 infective juveniles were washed several times in water and allowed to pass through a modified Baermann sieve. They were then collected and surface sterilised by immersion in 0.1% merthiolate for 1-2h followed by several washes in sterile water. Infective juveniles were then transferred onto agar surfaces and into buffer solutions in 0.1 ml of sterile water under sterile conditions. There were 50 infective juveniles per McCartney bottle and five replications for each pH/time combination and ten controls (nematodes in sterile water) at 23°C. Nematode survival was estimated at the end of two and four weeks by washing out the bottles with water and counting the nematodes microscopically.

vi) *Effect of Constant Temperatures on Nematode Development and Reproduction*

Last instar *G. mellonella* larvae were exposed individually to nematode infection within plastic specimen jars filled with moist fine sand. Dosages of either five *Heterorhabditis* or ten *Steinernema* infective juveniles were added to each specimen jar with 20 replications for each nematode/temperature combination. After insects and nematodes had been added to each jar, they were left for 16h (*S. glaseri*) or 48h (*S. feltiae, Heterorhabditis* sp. D1, *H. heliothidis* T327) at 23°C to allow infection. After this period, the dead *G. mellonella* larvae were placed in glass vials (diameter 2.5 cm, height 7.5 cm) containing 5 ml of 0.1% formalin. Each cadaver was supported above the formalin level by a piece of pleated filter paper (diameter 9.0 cm, height 4.5 cm) inserted into and placed at right angles to the 0.1% formalin. The tops of the glass vials were covered with parafilm "M" (Laboratory film, American Can Company) and the vials placed at the required constant temperatures.

Optimal temperatures for development and reproduction were assessed by determining the total number of infective-stage juveniles that emerged per cadaver. Cadavers which did not produce infective juveniles were dissected and examined microscopically in insect Ringer's solution.

vii) *Effect of Soil Type and Moisture on Behaviour and Survival of the Infective Juvenile Nematode*

Heterorhabditis sp. D1 and *S. glaseri* KG strain infective juveniles were obtained from *in vivo* cultures held at 23°C.

Prior to soil classification and analyses the different soil types were air dried, sieved through a 2 mm mesh sieve and stored in plastic bins.

Soil types were classified according to the system of Stace *et al.* (1968) and soil particle sizes were determined by dry sieving and sedimentation methods of Piper (1942). Organic matter content (organic carbon) was calculated using the rapid titration method of Walkley and Black (1934) and soil pH was determined from a mix of one part soil to five parts water using a calomel electrode in saturated KCl (Tucker and Beatty 1974). Soil surface areas were determined by the adsorption of water vapour method of Orchiston (1953) and a soil moisture characteristic curve was determined for each soil type using the pressure-plate method of McIntyre (1974).

Post-feeding third instar *L. cuprina* larvae were exposed individually to nematode infection within metal screwcap glass jars (diameter 4.0 cm, height 6.0 cm) filled to within 1.0 cm of the top with either fine sand, loamy sand or sandy clay loam (Krasnozem) of different moisture contents. Moisture contents* of the sand[a] and loamy sand[b] were established using an atomiser to spray on the required volume of water. The soil was then thoroughly mixed and placed into glass jars. Moisture contents of the Krasnozem[c] were established by placing air dried soil into the jars followed by the required amount of water; the jars were left for four to five days to obtain equilibration of water throughout the soil. Soil moisture contents were checked by oven drying samples at 105°C.

Dosages of 10^3 infective juveniles were introduced into a centrally placed well (diameter 1.0 cm, depth 2.0 cm) on 1.0 cm^2 pieces of filter paper and covered with soil. All jars were left for 16h at 28°C prior to the addition of *L. cuprina* larvae. One *L. cuprina* larva was placed on the soil surface and the lid screwed on after ensuring that the larva

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* Percentage moisture contents were:

(a) 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26.
(b) 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28.
(c) 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54.

had started to burrow. After 10 days at 28°C the sand and loamy sand were sieved in water while the Krasnozem was searched by hand for *L. cuprina* larvae or pupae. The larvae or pupae found were microscopically examined in insect Ringer's solution for nematode parasitization.

It was not feasible to examine simultaneously the three soil types, therefore experiments were divided into three groups; each group examined one soil type and consisted of 20 replicates for each moisture content/nematode combination, 20 nematode-free controls and 20 sand-filled specimen jars, moisture content 7% (pF = 1.3), each containing a dosage of 10^2 *Heterorhabditis* sp. DI infective juveniles.

RESULTS

i) *Low Temperature Immobilization of Infective Juvenile Nematodes*

The results of low temperature immobilization experiments are summarized in Table III. Very few *Heterorhabditis* species/strains were mobile at temperatures below 9°C and none were mobile below 7°C. *Heterorhabditis* sp. Q380 infective juveniles were immobile at 14°C.

All *Steinernema* spp. tested moved at 12°C and at least half of the *Steinernema* species/strains were mobile at temperatures below 7°C. *S. bibionis* strain N60 was still mobile at 1°C when touched with a mounted hair.

ii) *Effect of Temperature on the Parasitization of Insect Hosts by Infective Juvenile Nematodes*

The effects of temperature on nematode infectivity for *L. cuprina* larvae and subsequent nematode development and reproduction inside cadavers are presented in Figures 7 and 8 respectively. *Heterorhabditis* spp. did not parasitize *L. cuprina* larvae at temperatures below 8°C and

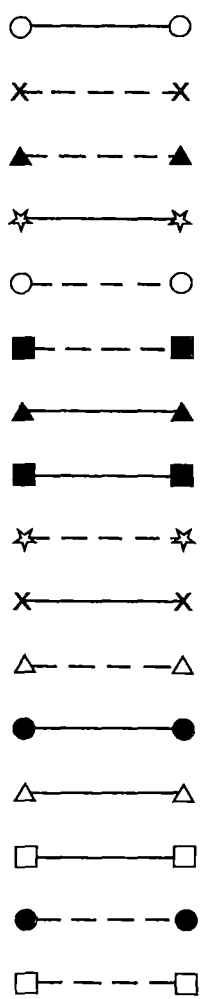
TABLE III

Immobilization of *Heterorhabditis* and *Steinernema* infective juvenile nematodes at low temperatures

Nematode	Strain	Temperature (°C) at which nematode is: Active	Barely Active	Inactive
Heterorhabditis sp.	Q380	16	15	14
Heterorhabditis sp.	D1	12	11	10
Heterorhabditis sp.	V16	12	10-11	9
H. bacteriophora	-	12	10-11	9
H. heliothidis	NZ	12	9-10	8
H. heliothidis	T327	10	7-8	6
H. heliothidis	NC	10	8-9	7
Heterorhabditis sp.	T310	10	8-9	7
Steinernema sp.	WI	14	11-12	10
Undescribed steinernematid	Q1	14	11-12	10
Steinernema sp.	WII	10	8-9	7
S. glaseri	KG	10	8-9	7
S. feltiae	Agriotos	8	6-7	5
S. bibionis	T335	4	3	2
S. kraussei	-	4	3	2
S. bibionis	N60	3	1-2	0

Fig. 7. Mortality of post-feeding third instar *Lucilia cuprina* strain AM-3 larvae exposed individually to dosages of 320 ($10^{2.5}$) heterorhabditid (A,B) and steinernematid (C,D) infective juvenile nematodes in 80 g sand (pF = 1.3) at various temperatures. Each point shows per cent mortality for 20 *L. cuprina* larvae.

Key:



Heterorhabditis sp. D1;
Heterorhabditis sp. Q380;
Heterorhabditis sp. V16;
Heterorhabditis sp. T310;
Heterorhabditis heliothidis T327;
Heterorhabditis heliothidis NC;
Heterorhabditis heliothidis NZ;
Heterorhabditis bacteriophora;
Steinernema sp. WI;
Steinernema sp. WII.
Steinernema bibionis N60;
Steinernema bibionis T335;
Steinernema feltiae Agriotos;
Steinernema glaseri KG;
Steinernema kraussei;
Undescribed steinernematid Q1;

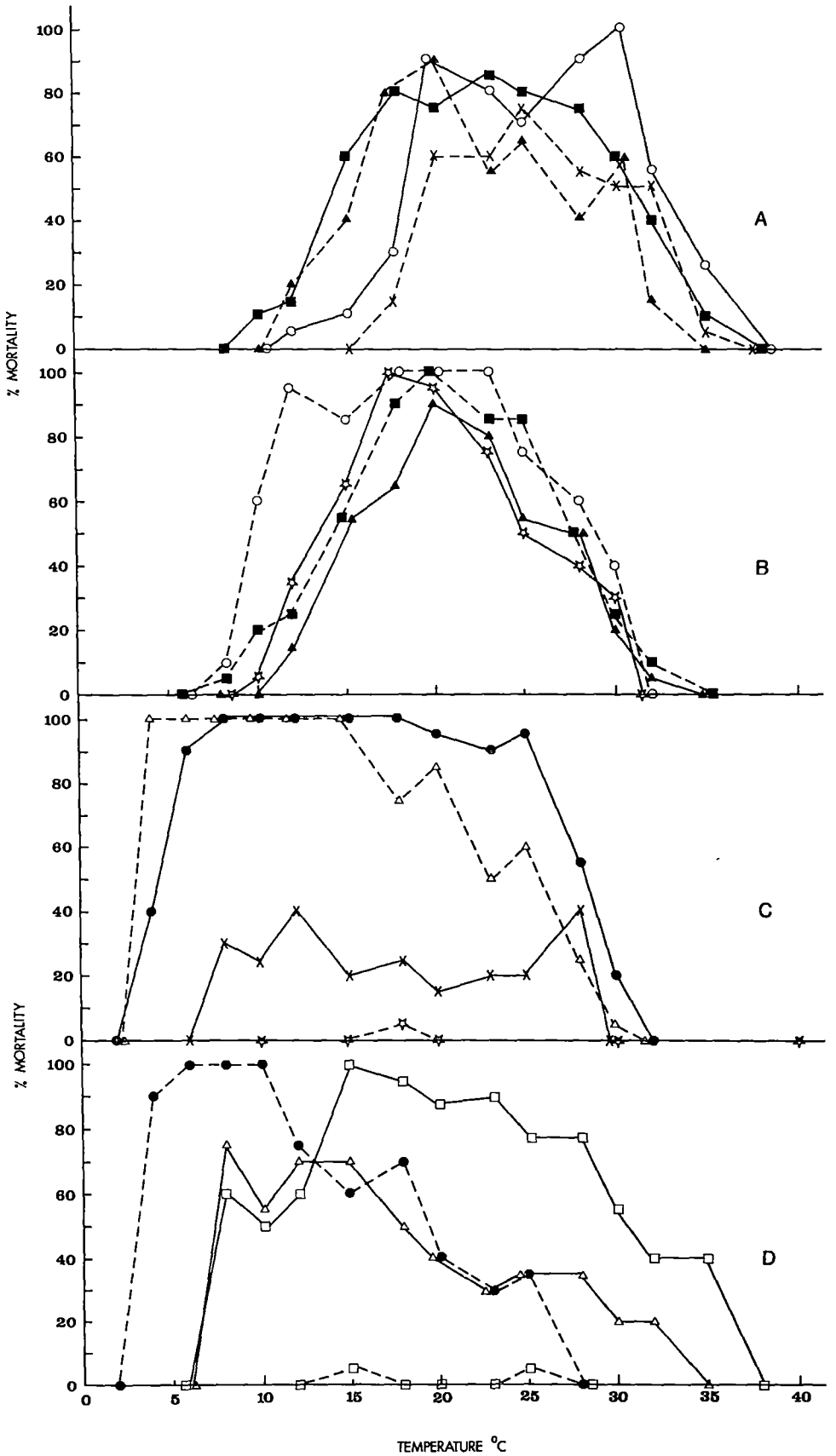

% MORTALITY
A
B
C
D
TEMPERATURE °C

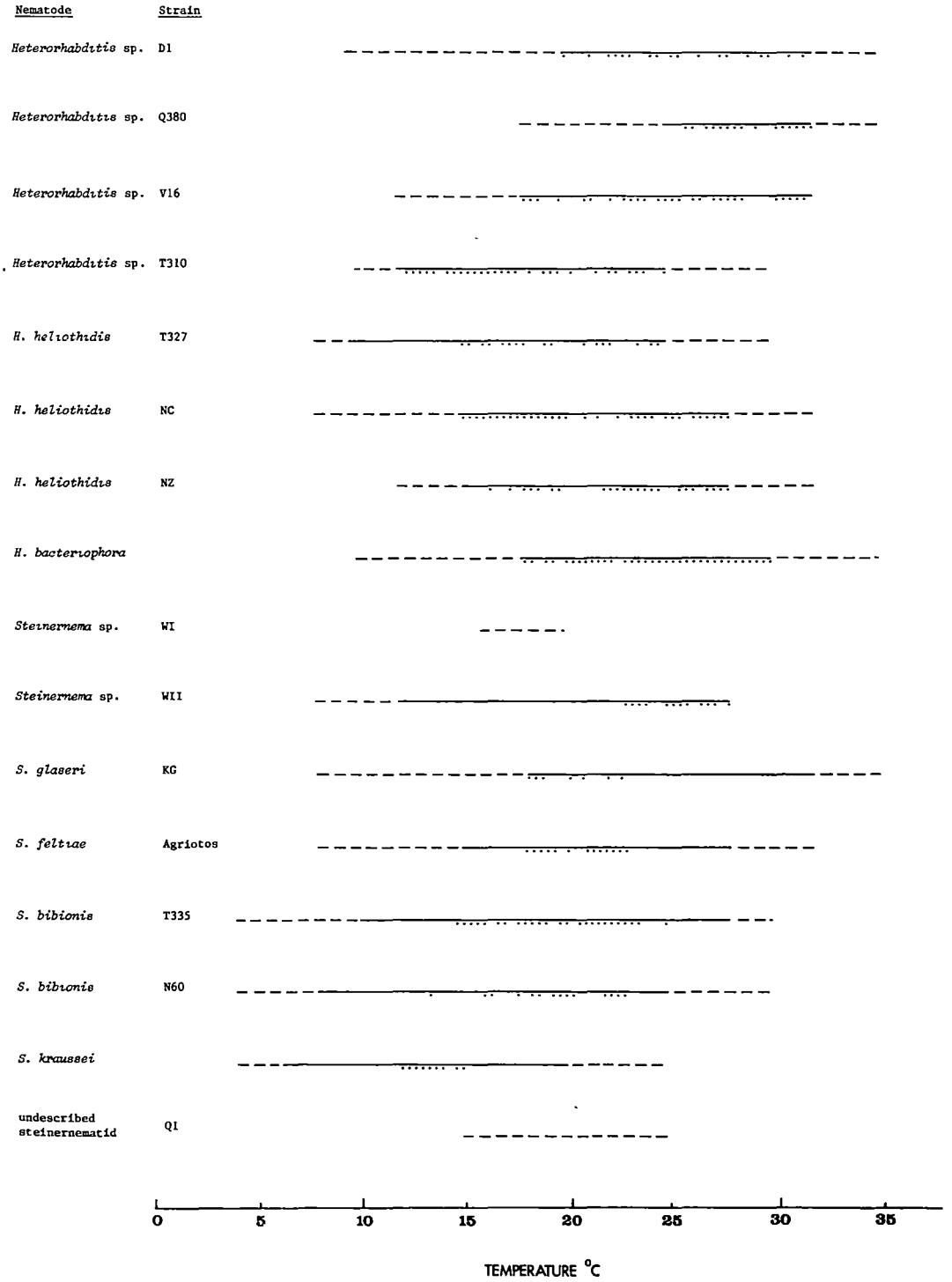
Fig. 8 Parasitization of post-feeding third instar *Lucilia cuprina* strain AM-3 larvae individually exposed to dosages of 320 heterorhabditid or steinernematid infective juvenile nematodes in 80 g sand (pF = 1.3) and subsequent nematode development, reproduction and production of infective juvenile nematodes at various temperatures.

Key:

— — — — — parasitization of *L. cuprina* larvae;

———————— nematode development and reproduction;

..... production of infective juvenile nematodes after four weeks.


Nematode
Strain
Heterorhabditis sp.
D1
Heterorhabditis sp.
Q380
Heterorhabditis sp.
V16
Heterorhabditis sp.
T310
H. heliothidis
T327
H. heliothidis
NC
H. heliothidis
NZ
H. bacteriophora
Steinernema sp.
WI
Steinernema sp.
WII
S. glaseri
KG
S. feltiae
Agriotos
S. bibionis
T335
S. bibionis
N60
S. kraussei
undescribed steinernematid
Q1
0
5
10
15
20
25
30
35
TEMPERATURE °C

only half of the heterorhabditids parasitized *L. cuprina* larvae at 10°C (Fig. 7a,b). In contrast, most of the steinernematids tested parasitized *L. cuprina* larvae at 8°C and some parasitized at 4°C (Fig. 7c,d). The majority of heterorhabditids parasitized *L. cuprina* larvae at 32°C whereas only *S. glaseri* KG strain and *S. feltiae* Agriotos strain parasitized *L. cuprina* larvae at temperatures above 30°C. None of the nematode species/strains tested parasitized *L. cuprina* larvae at temperatures below 4°C or above 35°C.

At temperatures above 25°C only the heterorhabditids and the steinernematid WII reproduced effectively, producing infective juvenile nematodes (Fig. 8). At temperatures below 25°C, steinernematids also reproduced effectively. However, only cadavers infected with either strain of *S. bibionis* produced infective juvenile nematodes over a relatively wide range of the temperatures tested (Fig. 8). The steinernematids WI and QI did not reproduce at any temperature tested.

In all cases, parasitization of *L. cuprina* larvae occurred over a greater range of temperature compared with the range of temperature allowing nematode development and reproduction (Fig. 8).

iii) *Quantitative Analysis of Insect Susceptibility at Different Temperatures*

The results of dosage*/mortality experiments for different temperatures are summarized in Tables IV and V and Figures 9-11. A detailed analysis of Table V is given in Appendix B. There was no significant difference between the results obtained for any nematode/temperature/dosage combination repeated at different times. LD_{50} and LD_{90} values were not calculated for *S. kraussei* at 28°C because mortality did not reach 50% at any dosage.

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* i.e. Dosage of nematodes into sand containing insects as specified in the methods on page 33.

TABLE IV

Effect of temperature on infectivity of *Heterorhabditis* spp. and *Steinernema* spp., for *Lucilia cuprina* strain AM-3 larvae in sand[a]

Nematode	Strain	18°C		23°C		28°C	
		LD_{50}	95% limits	LD_{50}	95% limits	LD_{50}	95% limits
Heterorhabditis sp.	DI	571	402-827	76	52-107	32	25-39
Heterorhabditis sp.	Q380	4,080	2,760-6,860	385	272-562	222	157-318
Heterorhabditis sp.	V16	50	30-78	145	92-227	239	167-342
H. bacteriophora		149	106-208	222	153-321	192	145-254
H. heliothidis	NC	45	31-63	87	64-116	170	123-233
H. heliothidis	NZ	331	223-497	125	88-175	183	138-240
H. heliothidis	T327	12	7-17	20	13-29	206	148-284
Steinernema glaseri	KG	45	30-65	41	29-57	88	63-120
S. bibionis	T335	20	14-28	41	30-54	155	129-186
S. bibionis	N60	99	70-136	336	221-519	23,900	10,600-75,000
S. feltiae	Agriotos	372	243-580	174	121-249	267	199-366
S. kraussei		213	149-303	1,710	1,040-3,160	*	

TABLE IV (Continued)

Nematode	Strain	18°C LD_{90}	18°C 95% limits	23°C LD_{90}	23°C 95% limits	28°C LD_{90}	28°C 95% limits
Heterorhabditis sp.	D1	7,820	4,450-17,100	941	590-1,750	609	446-881
Heterorhabditis sp.	Q380	46,700	22,300-143,000	8,370	4,510-19,300	5,920	3,270-13,100
Heterorhabditis sp.	V16	1,670	912-3,810	5,790	2,880-15,300	9,300	5,220-19,600
H. bacteriophora		1,610	1,020-2,970	3,580	2,080-7,460	4,190	2,700-7,250
H. heliothidis	NC	408	268-711	905	597-1,580	4,190	2,570-7,800
H. heliothidis	NZ	7,480	3,960-18,000	1,440	906-2,690	2,420	1,610-4,060
H. heliothidis	T327	107	69-189	239	152-434	4,970	3,030-9,300
Steinernema glaseri	KG	569	359-1,050	400	270-669	1,230	815-2,070
S. bibionis	T335	109	76-179	347	238-564	1,690	1,260-2,400
S. bibionis	N60	972	643-1,670	10,000	4,980-26,700	2.01×10^7	3.2×10^6-3.0×10^8
S. feltiae	Agriotos	11,900	5,750-33,000	4,320	2,500-8,840	5,890	3,440-11,820
S. kraussei		2,950	1,770-5,890	68,900	25,500-31,400	*	

[a] Individual larvae in 80 g sand, moisture content 7% (pF = 1.3). * Mortality did not reach 50% at any dosage.

TABLE V

Tests of coincidence between pairs of probit lines for differences in infectivity between various *Heterorhabditis* and *Steinernema* species/strains for post-feeding third instar *Lucilia cuprina* strain AM-3 larvae in sand[a] at various temperatures.

Comparison	Degrees of Freedom	Change in deviance 18°C	23°C	28°C
Steinernema bibionis T335 vs. *Steinernema bibionis* N60	2	55.6***	86.1***	408.0***
Heterorhabditis heliothidis T327 vs. *Heterorhabditis heliothidis* NC	2	28.5***	35.9***	0.71 ns
Heterorhabditis heliothidis T327 vs. *Heterorhabditis heliothidis* NZ	2	152.0***	49.5***	4.31 ns
Heterorhabditis heliothidis T327 vs. *Steinernema bibionis* T335	2	6.23*	8.66*	12.8**
Heterorhabditis heliothidis T327 vs. *Steinernema glaseri* KG	2	29.7***	7.7*	18.9***
Heterorhabditis sp. D1 vs. *Steinernema glaseri* KG	2	95.1***	8.15*	24.6***
Heterorhabditis sp. D1 vs. *Steinernema bibionis* T335	2	188.0***	10.7**	114.0***

ns not significant, $P > 0.05$
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
† details of analysis given in Appendix B
[a] individual *L. cuprina* larvae in 80g fine sand, moisture content 7% (pF 1.3).

Fig. 9 Dosage/mortality lines for various *Heterorhabditis* species/strains infecting post-feeding third instar *Lucilia cuprina* strain AM-3 larvae in sand (pF = 1.3) at various temperatures. Each point shows per cent mortality for 40 *L. cuprina* larvae.

Key: D1 = *Heterorhabditis* sp. D1; Q380 = *Heterorhabditis* sp. Q380; B = *Heterorhabditis bacteriophora*; V16 = *Heterorhabditis* sp. V16.

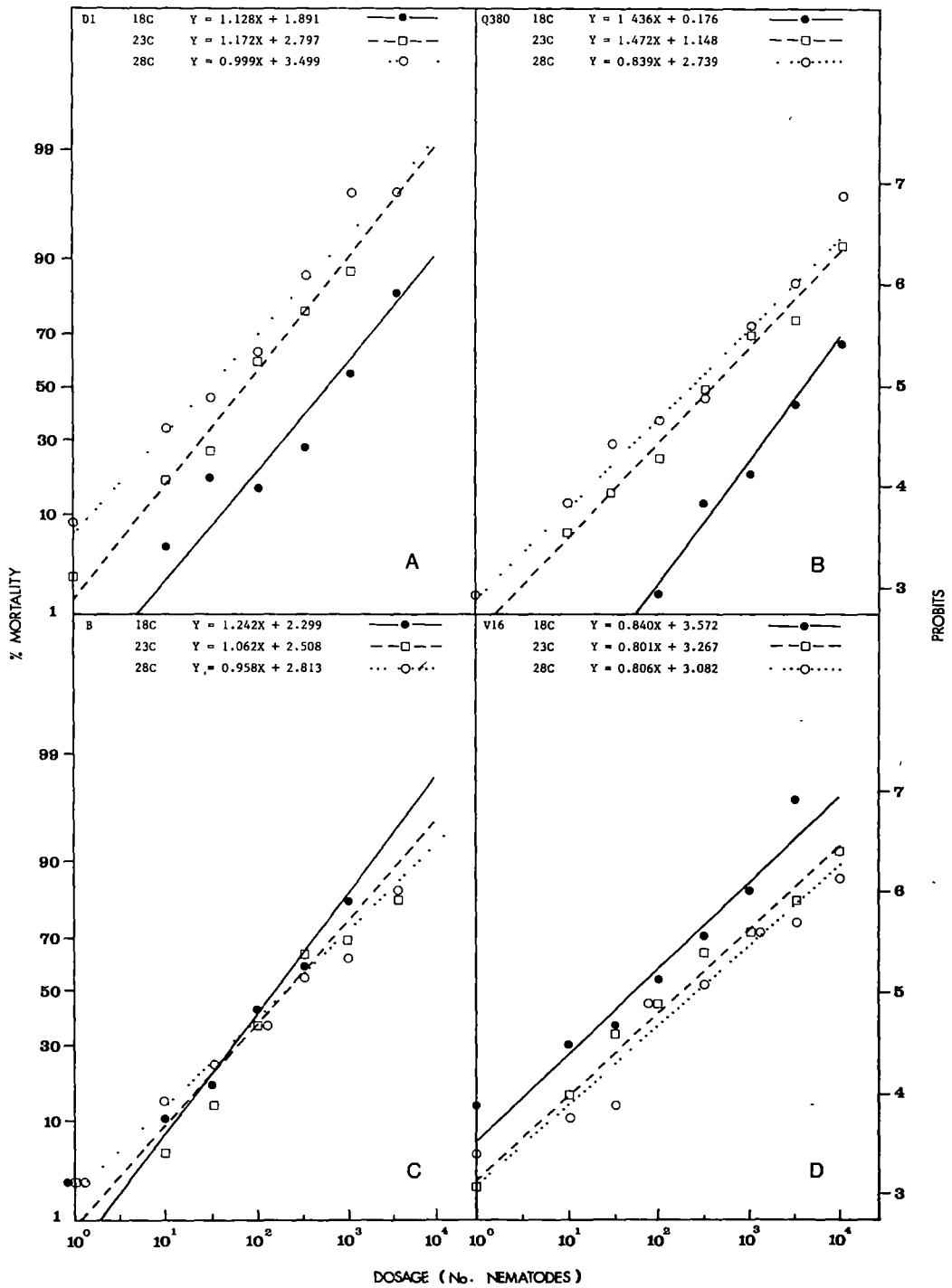

D1 18C Y = 1.128X + 1.891
23C Y = 1.172X + 2.797
28C Y = 0.999X + 3.499
Q380 18C Y = 1 436X + 0.176
23C Y = 1.472X + 1.148
28C Y = 0.839X + 2.739
B 18C Y = 1.242X + 2.299
23C Y = 1.062X + 2.508
28C Y = 0.958X + 2.813
V16 18C Y = 0.840X + 3.572
23C Y = 0.801X + 3.267
28C Y = 0.806X + 3.082
A
B
C
D
% MORTALITY
PROBITS
DOSAGE (No. NEMATODES)

Fig. 10 Dosage/mortality lines for various *Heterorhabditis heliothidis* strains (A-C) and *Steinernema kraussei* (D) infecting post-feeding third instar *Lucilia cuprina* strain AM-3 larvae in sand (pF = 1.3) at various temperatures. Each point shows per cent mortality for 40 *L. cuprina* larvae.

Key: NC = *Heterorhabditis heliothidis* NC; NZ = *Heterorhabditis heliothidis* NZ; T327 = *Heterorhabditis heliothidis* T327; K = *Steinernema kraussei*.

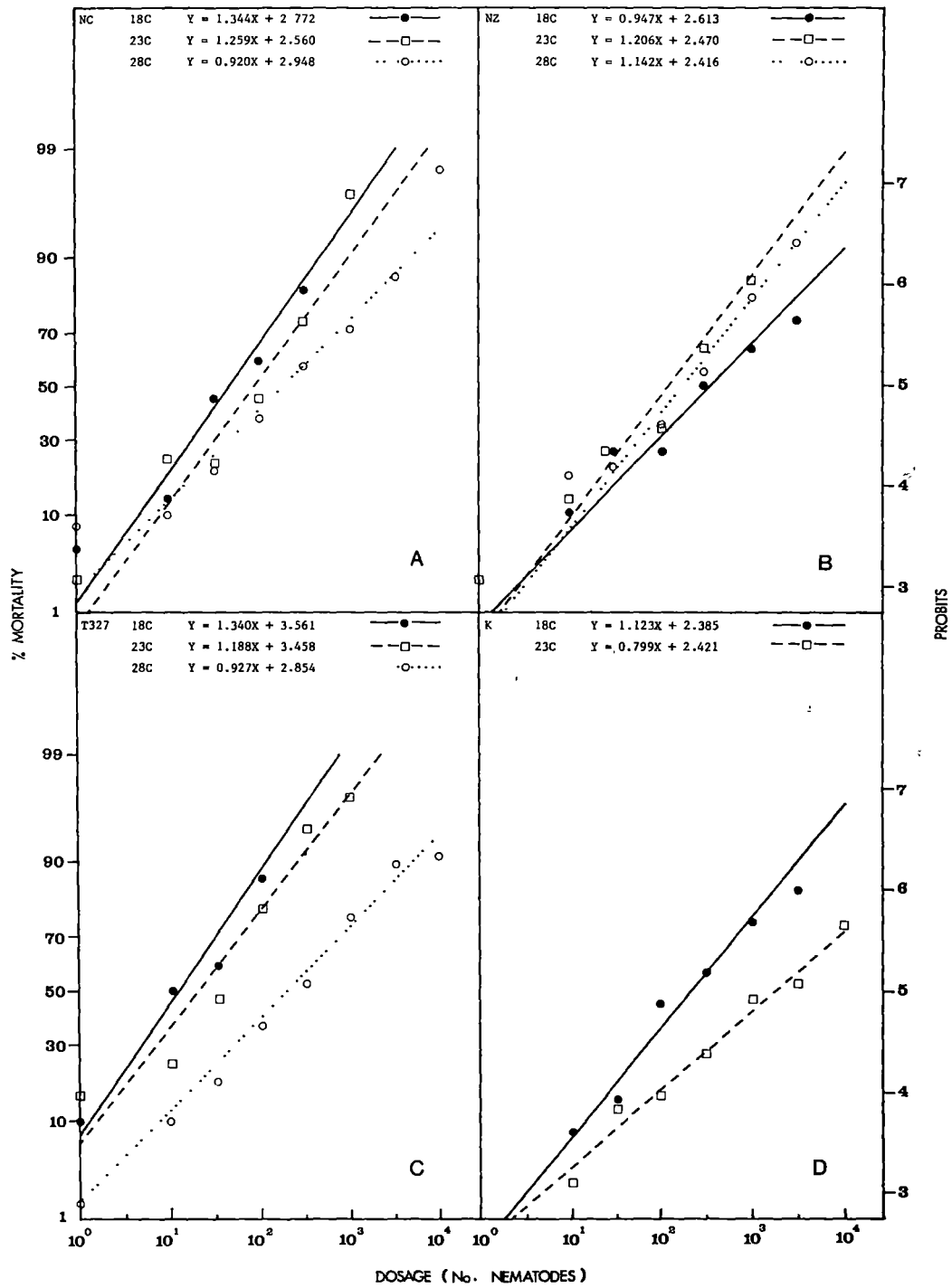
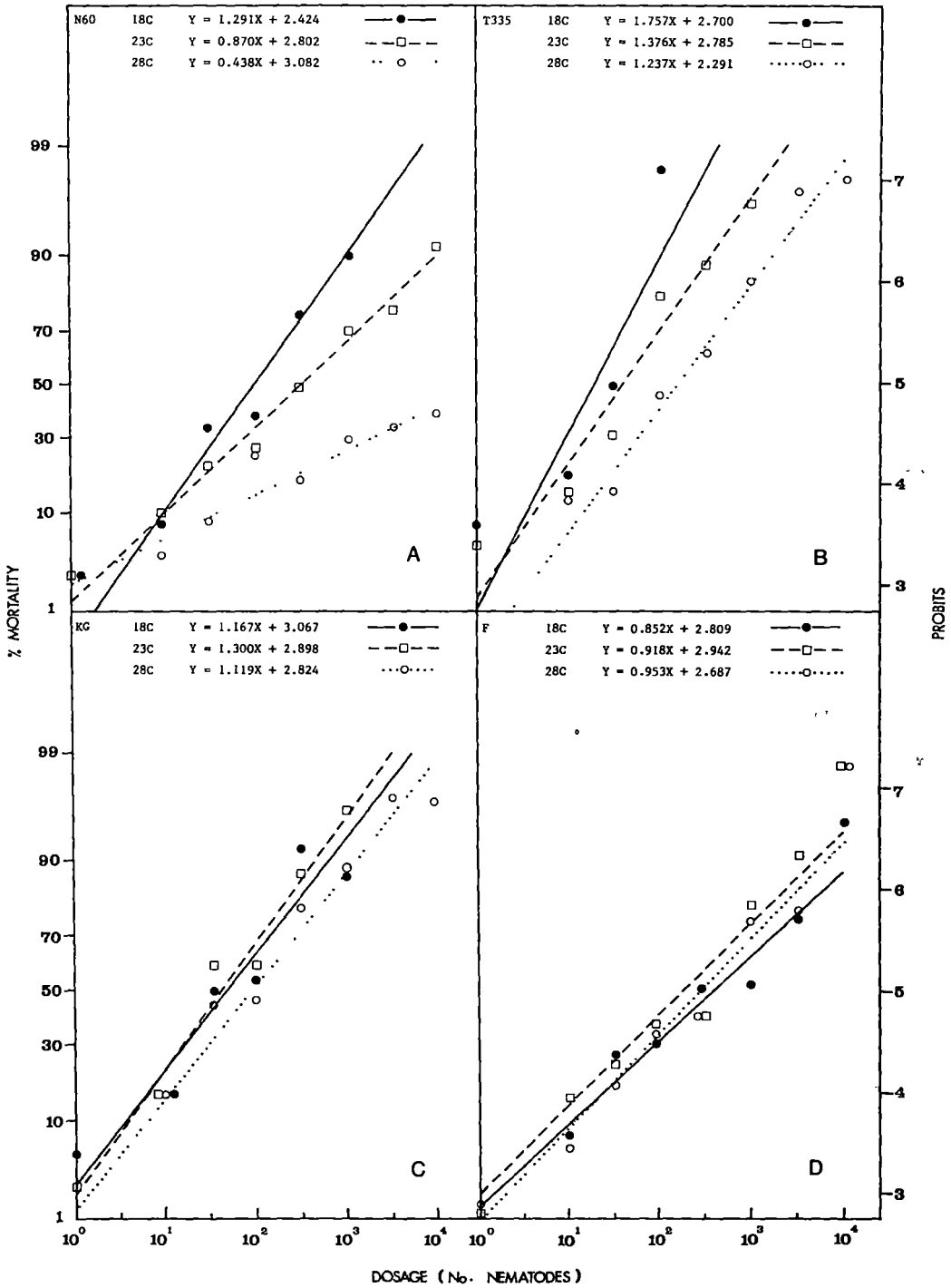

NC 18C Y = 1.344X + 2 772
23C Y = 1.259X + 2.560
28C Y = 0.920X + 2.948
NZ 18C Y = 0.947X + 2.613
23C Y = 1.206X + 2.470
28C Y = 1.142X + 2.416
T327 18C Y = 1.340X + 3.561
23C Y = 1.188X + 3.458
28C Y = 0.927X + 2.854
K 18C Y = 1.123X + 2.385
23C Y = 0.799X + 2.421
A
B
C
D
% MORTALITY
PROBITS
DOSAGE (No. NEMATODES)

Fig. 11 Dosage/mortality lines for various *Steinernema* species/strains infecting post-feeding third instar *Lucilia cuprina* strain AM-3 larvae in sand (pF 1.3) at various temperatures. Each point shows per cent mortality for 40 *L. cuprina* larvae.

Key: N60 = *Steinernema bibionis* N60; T335 = *Steinernema bibionis* T335; KG = *Steinernema glaseri* KG; F = *Steinernema feltiae* Agriotos.


N60 18C Y = 1.291X + 2.424
23C Y = 0.870X + 2.802
28C Y = 0.438X + 3.082
T335 18C Y = 1.757X + 2.700
23C Y = 1.376X + 2.785
28C Y = 1.237X + 2.291
KG 18C Y = 1.167X + 3.067
23C Y = 1.300X + 2.898
28C Y = 1.119X + 2.824
F 18C Y = 0.852X + 2.809
23C Y = 0.918X + 2.942
28C Y = 0.953X + 2.687
A
B
C
D
% MORTALITY
PROBITS
DOSAGE (No. NEMATODES)

The results show considerable differences between the 12 nematode species/strains, and more importantly between strains that are regarded as belonging to the same species. *S. bibionis* strain T335 was significantly better than *S. bibionis* strain N60 for all three temperatures tested and *H. heliothidis* strain T327 was significantly better than *H. heliothidis* strains NC and NZ at 18° and 23°C (Table V). However at 28°C, there was no significant difference between the three strains of *H. heliothidis*. Overall, *Heterorhabditis* sp. D1 was significantly better than any other nematode at 28°C (Tables IV,V), whereas *H. heliothidis* strain T327 was the most effective nematode at 18° and 23°C (Table V). At 18°C, *Heterorhabditis* sp. V16, *H. heliothidis* strains NC and T327, *S. kraussei* and *S. bibionis* strains T335 and N60 displayed optimum infectivity, whereas *H. heliothidis* strain NZ was more effective at 23°C. At 28°C, *Heterorhabditis* spp. D1 and Q380 were more effective than at lower temperatures. In contrast, there was no significant difference in the infectivity of *H. bacteriophora*, *S. glaseri* KG strain and *S. feltiae* Agriotos strain for *L. cuprina* between 18° and 28°C (Table IV and Figs. 9-11).

When *Steinernema* species infected *L. cuprina* larvae at 28°C, the majority of cadavers became fetid and the nematodes failed to reproduce effectively (i.e. infective juvenile nematodes were not produced). At 18° and 23°C, *Steinernema* developed and reproduced effectively in the majority of cadavers resulting from exposure to dosages of 10^1 and 10^3 infective juveniles, however *S. glaseri* reproduced only in cadavers that had been exposed to dosages of 10^1 and 10^2 infective juveniles. Cadavers resulting from exposure to higher dosages invariably became fetid and the nematodes failed to develop and reproduce.

The majority of *Heterorhabditis* species reproduced effectively in all cadavers at 28°C resulting from exposure to dosages of 10^0 to $10^{2.5}$ infective juveniles and there was occasional reproduction at dosages of 10^3. *H. heliothidis* strain T327 did not reproduce at any dosage at 28°C. At 23°C, all *Heterorhabditis* spp. reproduced in cadavers at dosage rates of 10^0 to $10^{2.5}$ infective juveniles. However at 18°C, *Heterorhabditis* spp. D1 and Q380 did not reproduce at any dosage in *L. cuprina* cadavers and *H. bacteriophora* reproduced only occasionally. The remaining heterorhabditids reproduced at 18°C in all cadavers resulting from dosages of 10^0 to $10^{2.5}$ infective juveniles except *H. heliothidis* strain T327 which reproduced only between dosages of 10^0 and 10^2 infective juveniles.

No production of infective juvenile nematodes occurred in cadavers resulting from dosages above $10^{2.5}$ *Heterorhabditis* infective juveniles at 18°, 23° and 28°C. At those dosages, the characterisitic pigmentation normally produced by the bacterial symbiont was absent and cadavers were invariably fetid.

iv) *Pupariation Times of L. cuprina Larvae for Different Temperatures*

The results are summarized in Table VI. The time taken for *L. cuprina* strain AM-3 larvae to pupariate in sand at different temperatures varied considerably. Although there was no significant difference between pupariation times at temperatures of 28° and 35°C, the time taken to pupariate increased dramatically at temperatures below 23°C. No pupariation was observed over a period of 28 days at 12°C.

TABLE VI

Pupariation of *Lucilia cuprina* strain AM-3 larvae at different temperatures in sand[a]

Temperature °C	Pupariation Time (Days) % Pupariation 5%	50%	95%
35	0.5	1	2
28	0.5	1.5	2
23	1.5	2 - 3	4 - 5
18	2 - 3	4 - 5	6 - 7
15	6 - 7	20	>28
12	>28	-	-

[a] Individual larvae in 80 g sand, moisture content 7% (pF = 1.3).

v) *The Effect of Temperature and pH on the Survival and Behaviour of Infective Juvenile Nematodes.*

Usually the survival of infective juvenile nematodes in sand increased generally with a decrease in temperature. However, although optimal survival of *H. heliothidis* strain T327 infective juveniles occurred at 10°C, survival at 28°C was greater than at 23°C (Fig. 12). Optimal survival of *Heterorhabditis* sp. D1 infective juveniles occurred at 15°C. At temperatures above and below 15°C, the survival of *Heterorhabditis* sp. D1 decreased rapidly to less than 10% of the original population within two weeks (Fig. 12). In contrast, the survival of *S. glaseri* KG strain infective juveniles over 32 weeks was apparently independent of temperature (Fig. 13).

Declines in the survival of *Heterorhabditis* sp. D1 similar to that in sand were observed after infective juveniles had been placed in peat, vermiculite, loamy sand and heat sterilized sand at similar moisture potentials and left for two weeks at 23°C. Furthermore, a similar mortality of *Heterorhabditis* sp. D1 infective juveniles at high density was observed after two weeks at 23°C (one million nematodes placed in 3,230 c.c. of sand [pF 1.3]). *Heterorhabditis* sp. D1 infective juveniles newly emerged from *G. mellonella* cadavers held in sand at 23°C also survived for similar periods of time.

Within two weeks at 28°C and 23°C, *Heterorhabditis* sp. D1, *H. heliothidis* strain T327 and *S. feltiae* Agriotos strain infective juveniles appeared almost transparent. Similarly *S. glaseri* KG strain infective juveniles became relatively transparent but only after eight weeks at 28°C. At 10°C, there was no noticeable decrease in the body contents of infective juveniles of *S. glaseri* even after 32 weeks.

Fig. 12 The effect of time on survival of *Heterorhabditis* sp. D1 and *Heterorhabditis heliothidis* T327 infective juvenile nematodes in sand (pF = 1.3) at 28° (A), 23° (B), 15° (C) and 10°C (D), and their infectivity for post-feeding third instar *Lucilia cuprina* strain AM-3 larvae in sand (pF = 1.3) at 23°C. The discontinuous line represents the number of infective juvenile nematodes recovered using a modified Baermann sieve. Each survival and per cent mortality point is the result of four and 20 replications respectively.

Key:

Percent mortality of *L. cuprina* larvae	No. of nematodes recovered from sand
○ , ●	*Heterorhabditis* sp. D1
△ , ▲	*Heterorhabditis heliothidis* T327.

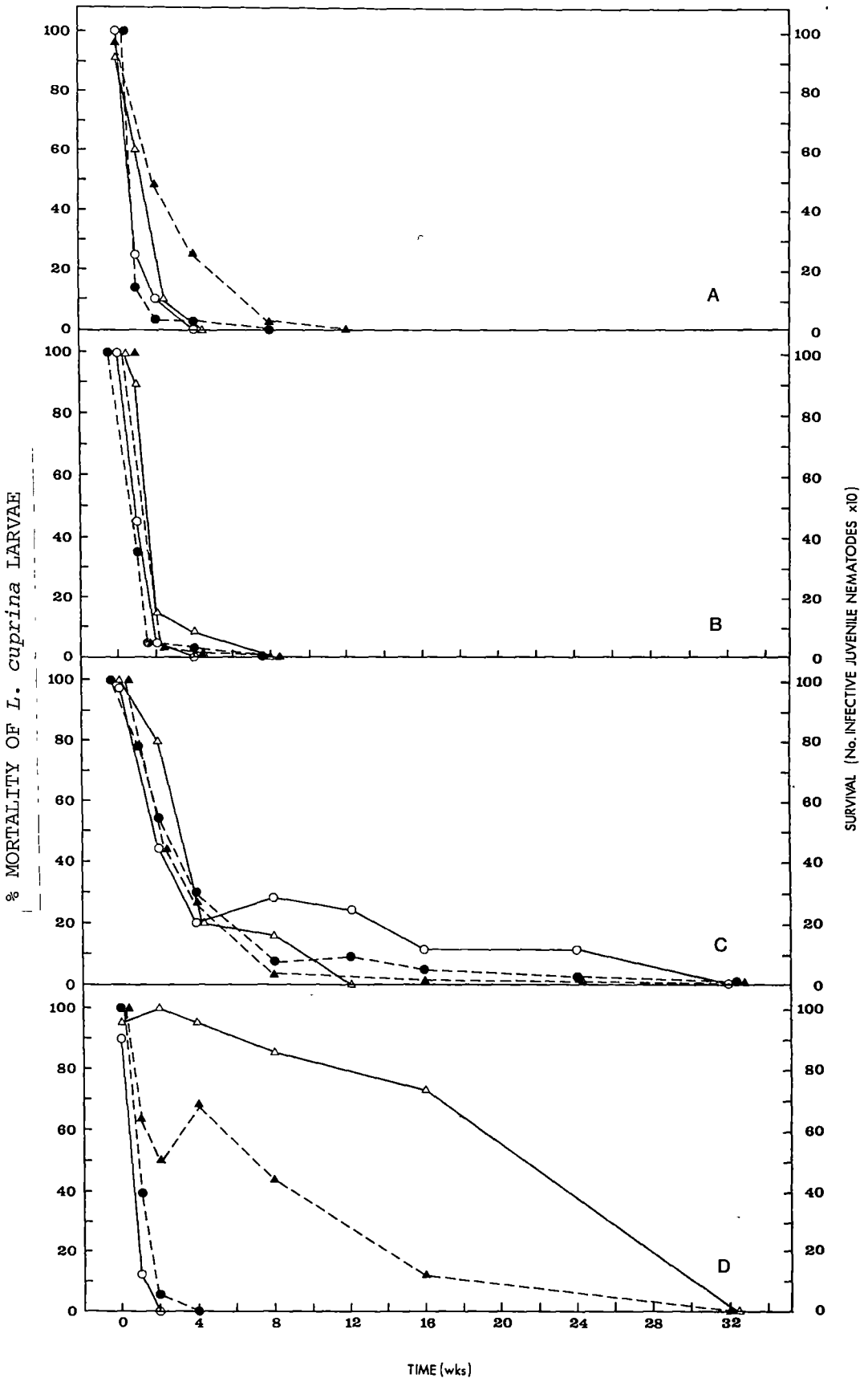
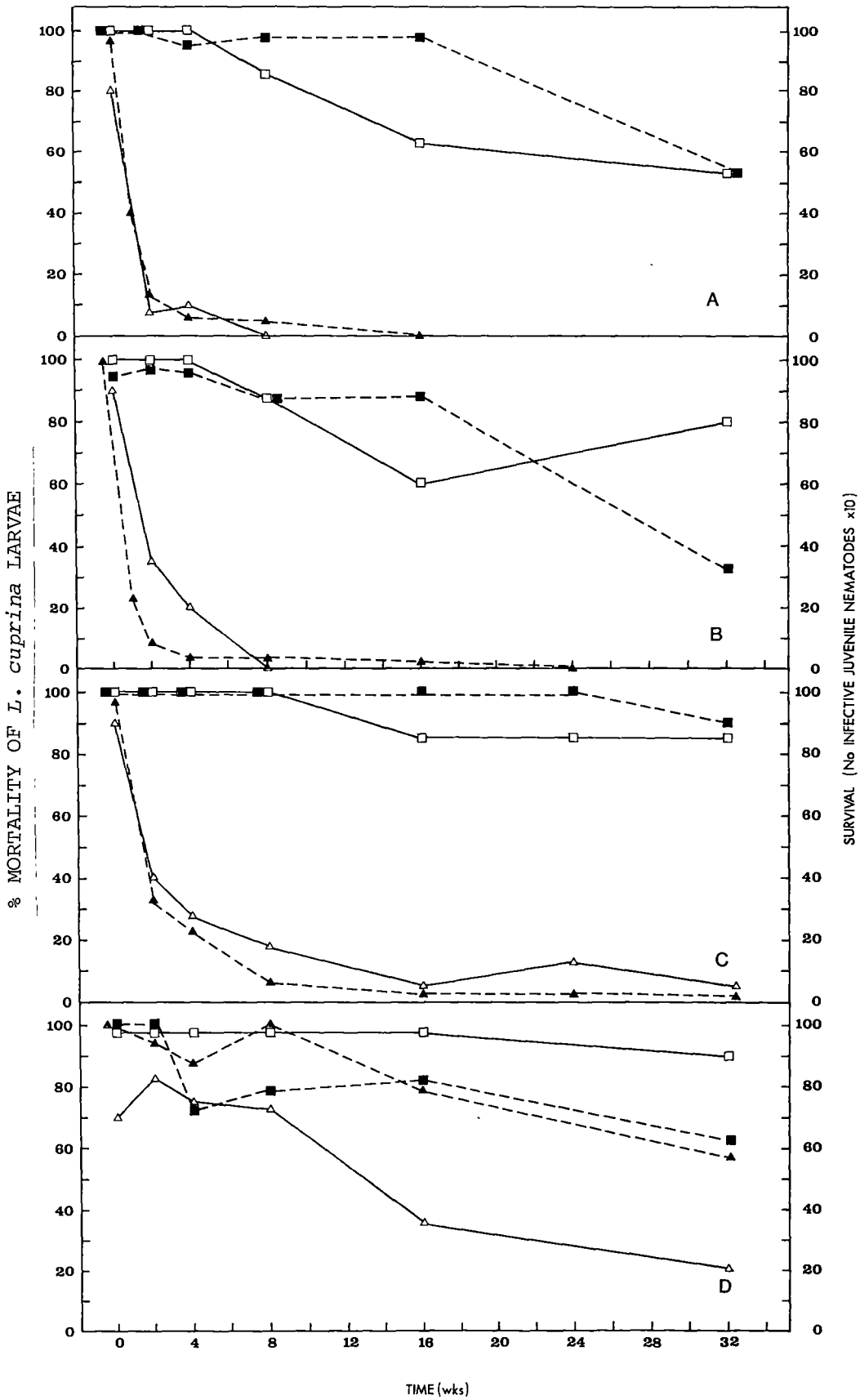

% MORTALITY OF L. cuprina LARVAE
SURVIVAL (No. INFECTIVE JUVENILE NEMATODES x10)
TIME (wks)
A
B
C
D
0
4
8
12
16
20
24
28
32
0
20
40
60
80
100

Fig. 13 The effect of time on survival of *Steinernema glaseri* KG strain and *Steinernema feltiae* Agriotos strain infective juvenile nematodes in sand (pF = 1.3) at 28° (A), 23° (B), 15°(C) and 10° (D), and their infectivity for post-feeding third instar *Lucilia cuprina* strain AM-3 larvae in sand (pF = 1.3) at 23°C. The discontinuous line represents the number of infective juvenile nematodes recovered using a modified Baermann sieve. Each survival and per cent mortality point is the result of four and 20 replications respectively.

Key:

Percent mortality of *L. cuprina* larvae	No. of nematodes recovered from sand
□ , ■	*Steinernema glaseri* KG
△ , ▲	*Steinernema feltiae* Agriotos


% MORTALITY OF L. cuprina LARVAE
SURVIVAL (No INFECTIVE JUVENILE NEMATODES x10)
TIME (wks)
A
B
C
D
0
4
8
12
16
20
24
28
32
20
40
60
80
100

Unlike the heterorhabditids and *S. feltiae* Agriotos strain, infective juveniles of *S. glaseri* were often observed in a non-moving coiled position when washed from the sides of sand-filled specimen jars (Fig. 14).

Nematode activity (expressed as per cent parasitization of *L. cuprina* larvae) was closely correlated with nematode survival and age (Figs. 12 and 13). Although *Heterorhabditis* sp. D1 and *H. heliothidis* strain T327 infective juveniles at 28°C and 23°C respectively, failed to parasitize *L. cuprina* larvae after two weeks, final instar *G. mellonella* larvae were parasitized by infective juveniles that had been kept in sand for up to 16 weeks. After surviving eight weeks at 15°C, *H. heliothidis* strain T327 infective juveniles did not parasitize *L. cuprina* larvae (Fig. 12) but continued to infect *G. mellonella* larvae after infective juveniles had been in sand for 32 weeks. Similarly, at 23°C and 28°C *L. cuprina* larvae were not parasitized by *S. feltiae* Agriotos strain infective juveniles that had been in sand for eight weeks (Fig. 13) but continued to parasitize *G. mellonella* larvae until the infective juveniles were 16 weeks of age. In contrast, *S. glaseri* KG strain infective juveniles parasitized *L. cuprina* larvae throughout 32 weeks at each temperature tested (Fig. 13).

Nematode development and reproduction occurred generally in all *G. mellonella* larvae parasitized by infective juveniles aged between one and 32 weeks at each temperature tested. The one exception was when *G. mellonella* larvae were parasitized by *Heterorhabditis* sp. D1 that had been in sand for one week at 10°C. After two weeks at 23°C, cadavers were found to contain dead first generation adult nematodes.

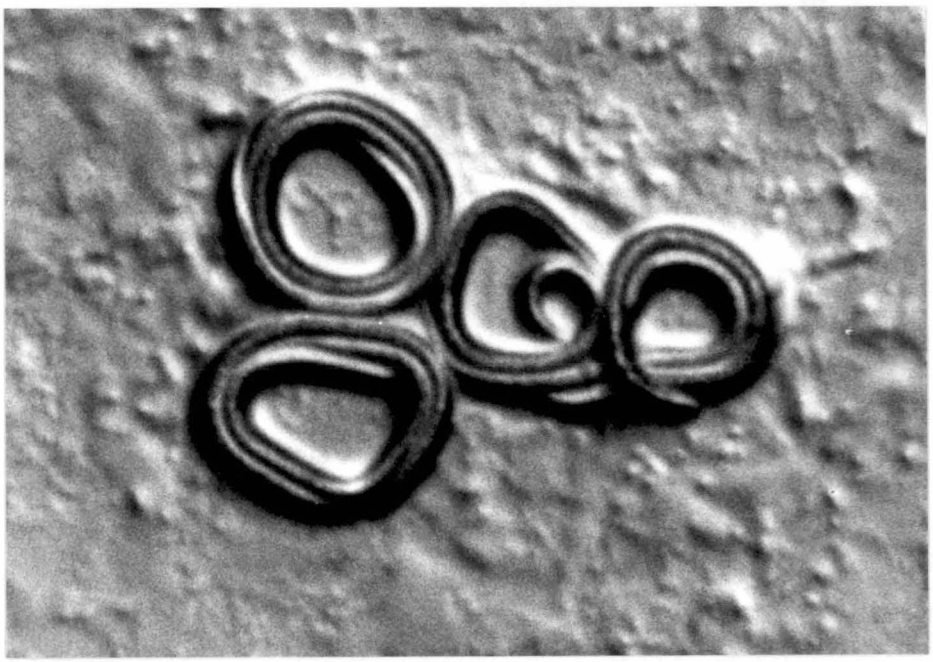


Fig. 14 (70x) *Steinernema glaseri* strain KG infective juvenile nematodes in a coiled inactive position after two weeks on a 1.5% sterile purified agar surface at 23°C.

Nematode activity was also measured in terms of the time taken to recover active infective juveniles from sand using a modified Baermann sieve (details of analysis are given in Appendix C). There was no difference in the activity of *S. glaseri* KG strain infective juveniles throughout 32 weeks at each temperature tested, 'active' nematodes being recovered within 24 to 48h. In contrast, the activity of *Heterorhabditis* sp. D1, *H. heliothidis* strain T327 and *S. feltiae* Agriotos strain declined rapidly with time, often three to five days being required for recovery of 'active' nematodes.

The results of aerating *Heterorhabditis* sp. D1 and *S. feltiae* Agriotos strain infective juveniles in water are summarized in Table VII and Figures 15 and 16. Although there was a steady decline in the survival of *S. feltiae* Agriotos strain infective juveniles, there was no significant difference in the infectivity of surviving infective juveniles for *L. cuprina* larvae from 0-18 weeks at 23°C (Table VII, Figs. 15b and 16b). However, the infectivity of surviving infective juveniles of *Heterorhabditis* sp. D1 for *L. cuprina* larvae decreased significantly ($p < .001$) together with a corresponding decline in the survival of infective juvenile nematodes over 16 weeks at 23°C (Table VII, Figs. 15a and 16a).

The results of the effects of pH on the survival of *Heterorhabditis* sp. D1 infective juveniles are summarized in Table VIII.

vi) *Effect of Constant Temperatures on Nematode Development and Reproduction*

The results of the effect of temperature on the production and emergence of infective juvenile nematodes from final instar *G. mellonella* larvae are summarized in Figures 17-19.

Fig. 15 The effect of time on the survival of *Heterorhabditis* sp. D1 (A) and *Steinernema feltiae* Agriotos (B) infective juvenile nematodes (aerated in water at a density of 8,000/ml at 23°C) and their infectivity for post-feeding third instar *Lucilia cuprina* strain AM-3 larvae after individual exposure to dosages of 10^3 *Heterorhabditis* sp. D1 or *S. feltiae* Agriotos in sand (pF = 1.3) at 23°C. Each survival and per cent mortality point is the result of three and 20 replications respectively. The discontinuous line represents per cent mortality of *L. cuprina* over time. The vertical lines represent standard error.

Key:

Percent mortality of *L. cuprina* larvae	Percent survival of nematodes
△ ,	▲ *Heterorhabditis* sp. D1
□ ,	■ *Steinernema feltiae* Agriotos

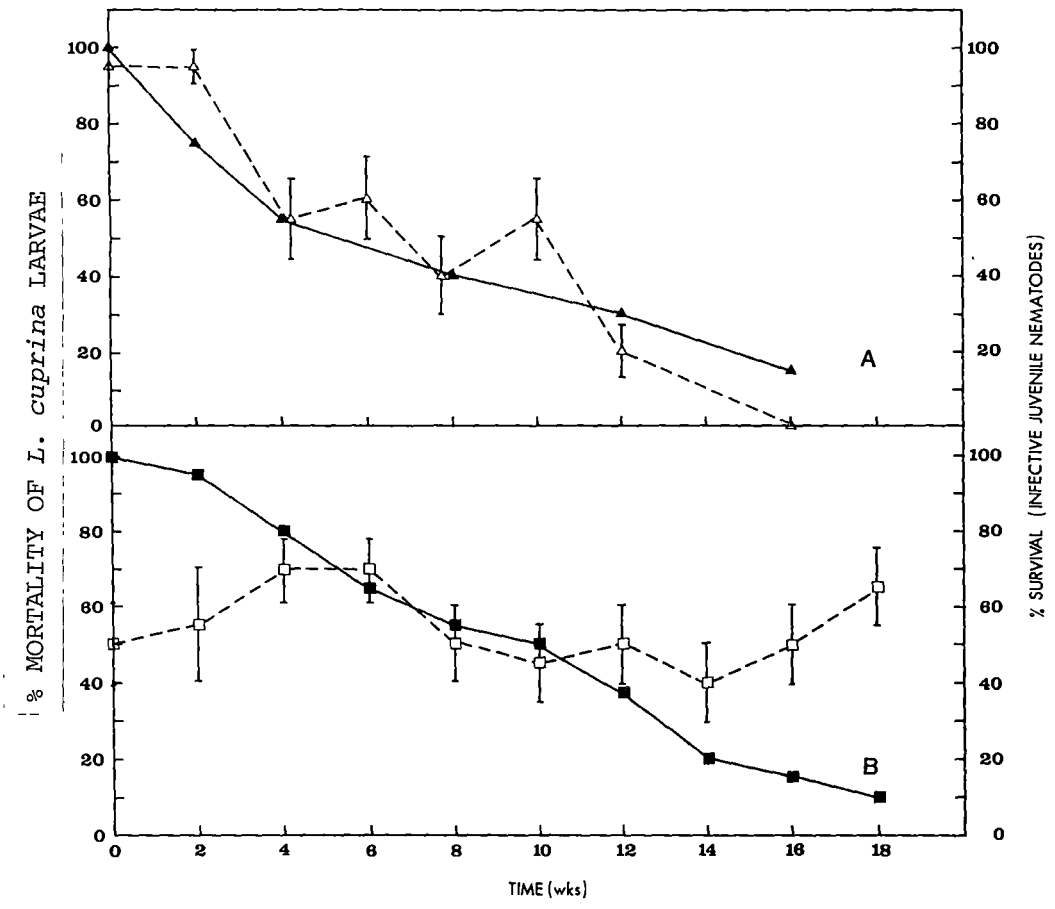

% MORTALITY OF L. cuprina LARVAE
% SURVIVAL (INFECTIVE JUVENILE NEMATODES)
TIME (wks)
A
B
0
20
40
60
80
100
0
2
4
6
8
10
12
14
16
18

Fig. 16 The effect of age of *Heterorhabditis* sp. D1 (A) and *Steinernema feltiae* Agriotos strain (B) infective juvenile nematodes (aerated in water at 8,000/ml at 23°C from 0-18 weeks) and their infectivity for post-feeding third instar *Lucilia cuprina* strain AM-3 larvae in sand (pF = 1.3) at 23°C. Each point represents the log number of infective juvenile nematodes calculated to give a 50% mortality of a sample population of *L. cuprina* larvae. Vertical lines represent the 95% confidence limits. Calculations were made on actual numbers and then subsequently converted to logs for plotting.

* Upper limit approaches log LD_{50} = 27.0.

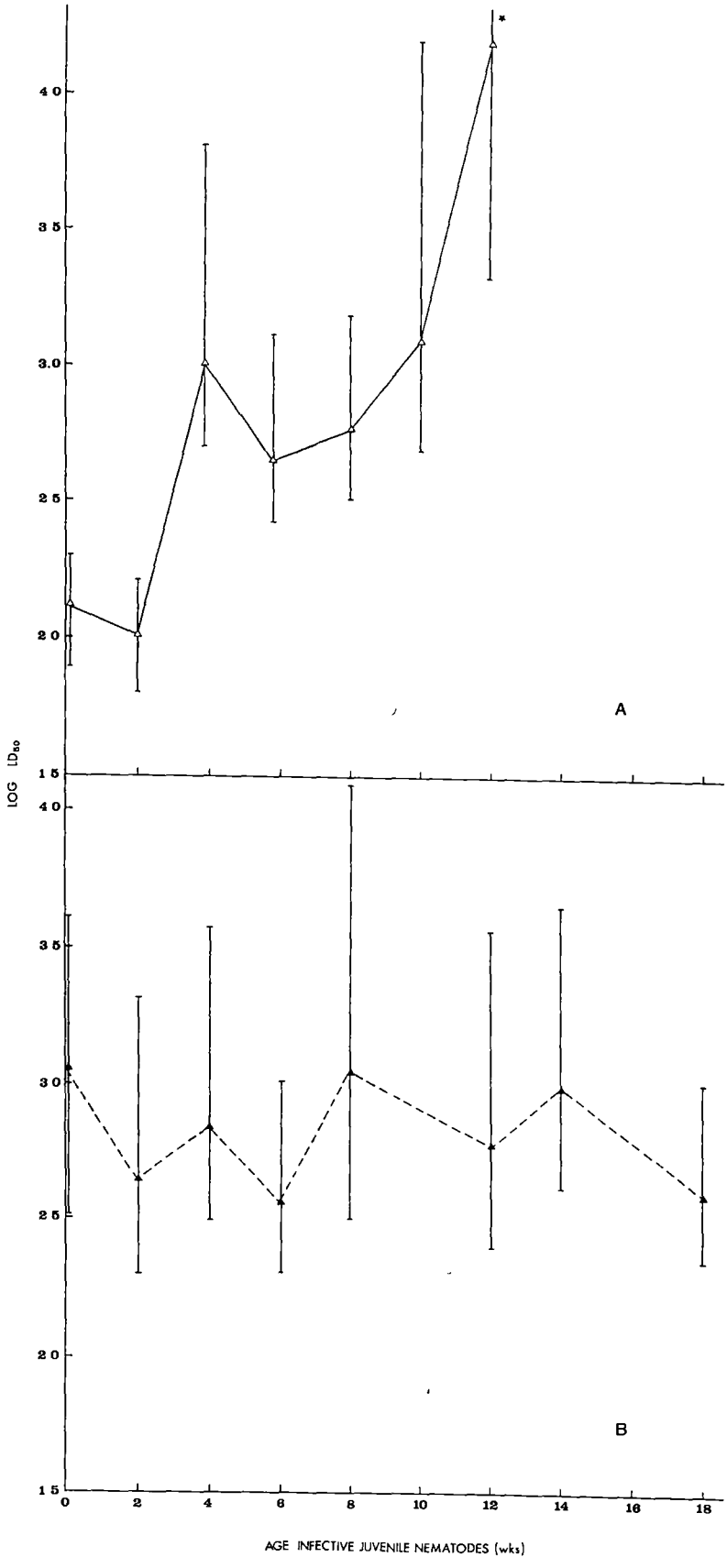

LOG LD50
4.0
3.5
3.0
2.5
2.0
1.5
A
B
0
2
4
6
8
10
12
14
16
18
AGE INFECTIVE JUVENILE NEMATODES (wks)

TABLE VII

Tests of coincidence between probit lines for differences in the infectivity of *Heterorhabditis* sp. D1 and *Steinernema feltiae* Agriotos strain infective juvenile nematodes (aerated in water at a density of 8,000/ml from 0-18 weeks at 23°C) for post-feeding third instar *Lucilia cuprina* strain AM-3 larvae larvae in sand[a] at 23°C.

Data Set	Common Line		Individual Line		Comparison	
	Degrees of Freedom	Deviance	Degrees of Freedom	Deviance	Degrees of Freedom	Changes in Deviance
Steinernema feltiae Agriotos strain	48	75.16	30	50.13	18	25.03 ns
Heterorhabditis sp. D1	33	130.50	21	24.77	12	105.70 ***

a individual *L. cuprina* larvae in 80 g sand, moisture content 7% (pF = 1.3).
*** $P < 0.001$.

TABLE VIII

The effect of pH on survival of *Heterorhabditis* sp. D1 infective juvenile nematodes at 23°C.

Medium	Replicates	TWO WEEKS Mean no. ± s.d. live nematodes[a] pH 4.5	5.0	5.5	6.0	6.5	FOUR WEEKS Mean No. ± s.d. live nematodes[a] pH 4.5	5.0	5.5	6.0	6.5
Buffered agars	5	-	9±4	17±12	34±6	32±6	-	0	3±1	13±6	7±3
Buffered solutions	5	42±5	47±1	43±8	46±3	41±1	34±4	40±6	33±6	42±4	30±8
Agar control	5			21±15					1±1		
Water control	5				48±2					41±3	

[a] A total of 50 infective juvenile nematodes per replicate.

At temperatures below 10°C, *H. heliothidis* strain T327, *S. glaseri* KG strain and *S. feltiae* Agriotos strain did not develop and no live nematodes were recovered at the end of 14 days. At 10°C, *S. glaseri* KG strain and *S. feltiae* Agriotos strain moulted into the fourth larval stage (L4) and *H. heliothidis* strain T327 developed into first generation adults, but died after four weeks without further development. At 12°C, a few infective juveniles emerged from approximately 10% of cadavers infected with *S. glaseri* KG strain and *H. heliothidis* strain T327 after eight weeks, and after 10 weeks from cadavers infected with *S. feltiae* Agriotos strain (Fig. 18). At 15°C, infective juvenile nematodes emerged from all *G. mellonella* larvae infected with *H. heliothidis* strain T327 or *S. feltiae* Agriotos strain and from about 80% of *G. mellonella* larvae infected with *S. glaseri* KG strain.

Heterorhabditis sp. D1 did not reproduce at 18°C and only developed partially to L4 stages within nine days after infection of the host. Between 20° and 32°C, *Heterorhabditis* sp. D1 and *S. glaseri* KG strain developed and reproduced, giving maximal production of infective juveniles at 30° and 23°C respectively (Fig. 17). *S. glaseri* KG strain infective juveniles and many pre-infective juveniles emerged after four days and *Heterorhabditis* sp. D1 infective juveniles emerged after 10 days at 28°C (Fig. 18). At 35°C, *S. glaseri* KG strain developed to first generation adults but failed to reproduce while *Heterorhabditis* sp. D1 did not develop at all.

Maximal reproduction of *H. heliothidis* strain T327 and *S. feltiae* Agriotos strain occurred at 20° and 23°C respectively (Fig. 17). Above 25°C, there was a decline in fecundity and at 28°C *H. heliothidis* strain T327 developed only to first generation adults. *S. feltiae* Agriotos

Fig. 17 Total number of infective juvenile nematodes produced from final instar *Galleria mellonella* larvae at various temperatures. *G. mellonella* larvae were infected with either five heterorhabditid or ten steinernematid infective juvenile nematodes in sand (pF = 1.3) at 23°C. Cadavers were then placed on moist filter paper at the required temperatures. Each point represents the mean number of infective juvenile nematodes produced from 20 *G. mellonella* larvae and the vertical lines represent the 95% confidence limits.

Key:

●——● *Heterorhabditis* sp. D1;

○- - - -○ *Heterorhabditis heliothidis* T327;

▲——▲ *Steinernema glaseri* KG;

△- - - -△ *Steinernema feltiae* Agriotos.

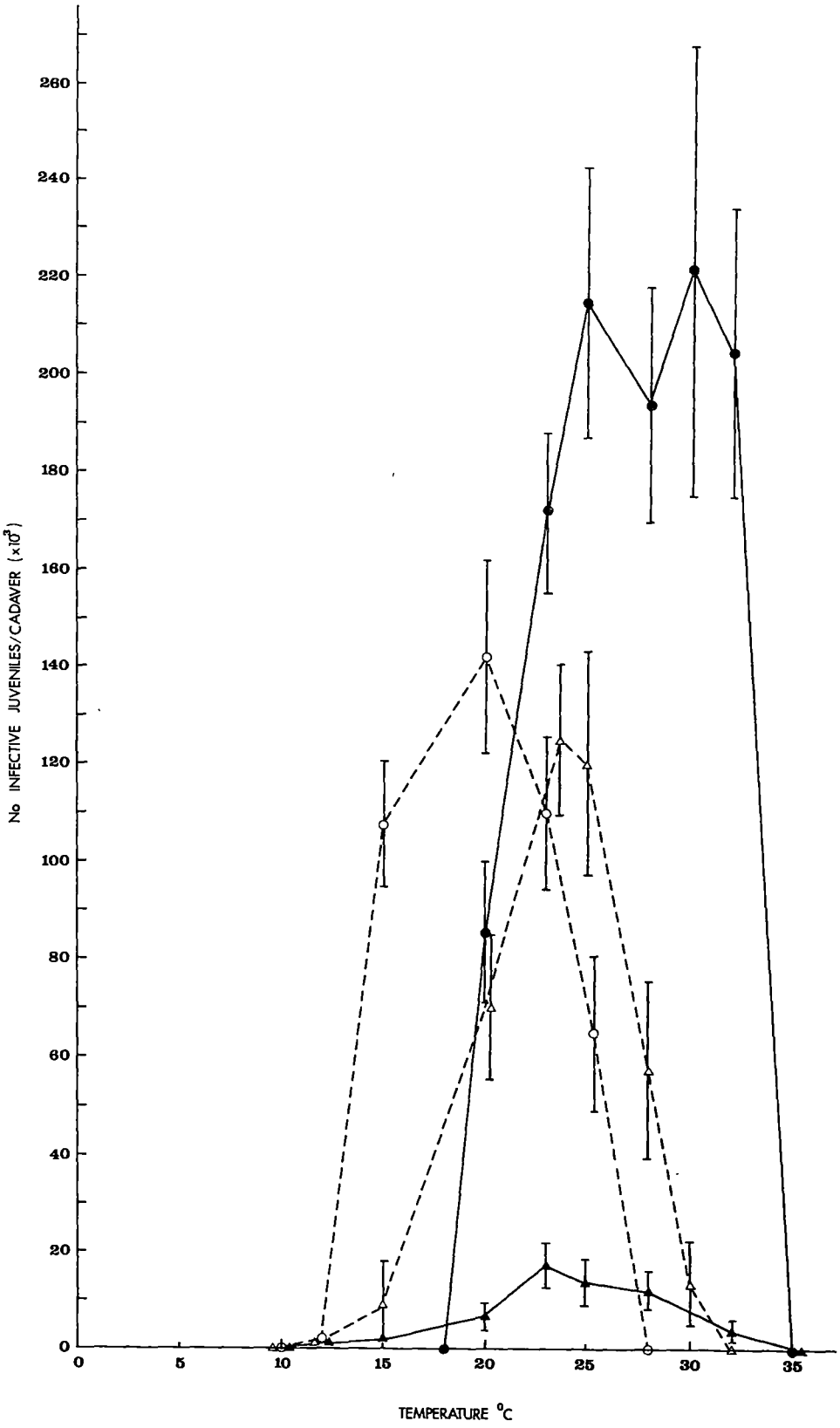
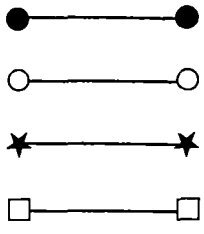

No INFECTIVE JUVENILES/CADAVER (×10³)
260
240
220
200
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
TEMPERATURE °C

Fig. 18 Time taken between infection of host and emergence of various *Heterorhabditis* and *Steinernema* infective juvenile nematodes from final instar *Galleria mellonella* cadavers at various temperatures. *G. mellonella* larvae were initially infected with either five heterorhabditid or ten steinernematid infective juvenile nematodes in sand (pF = 1.3) at 23°C. Cadavers were then placed on moist filter paper at the required temperatures.

Key:



Heterorhabditis sp. D1;

Heterorhabditis heliothidis T327;

Steinernema feltiae Agriotos;

Steinernema glaseri KG.

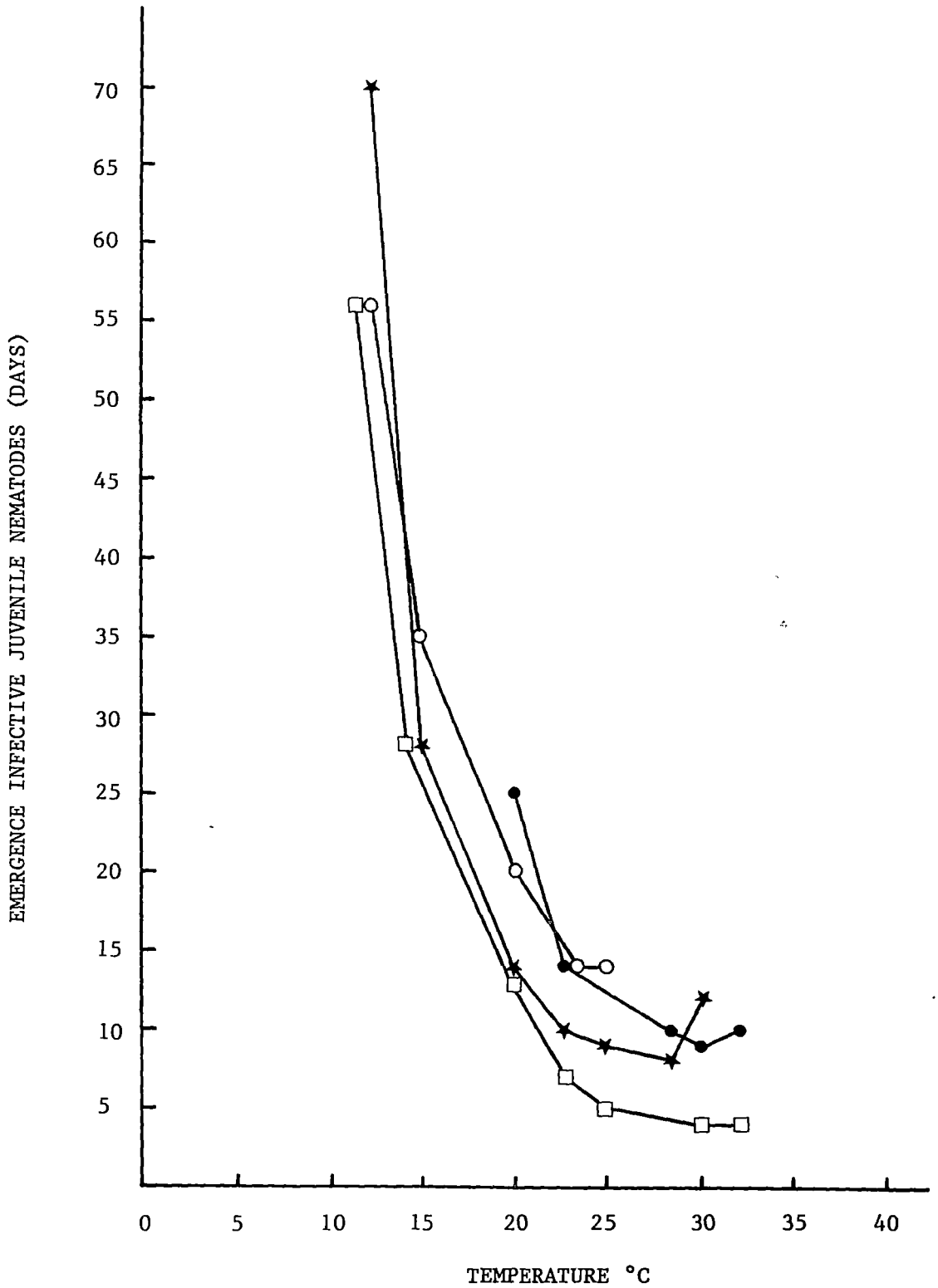

EMERGENCE INFECTIVE JUVENILE NEMATODES (DAYS)
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
5
10
15
20
25
30
35
40
TEMPERATURE °C

Fig. 19. The reciprocal of time taken between infection of host and emergence of various *Heterorhabditis* and *Steinernema* infective juvenile nematodes from final instar *Galleria mellonella* cadavers at various temperatures. The slope of the line indicates the average rate of nematode development.

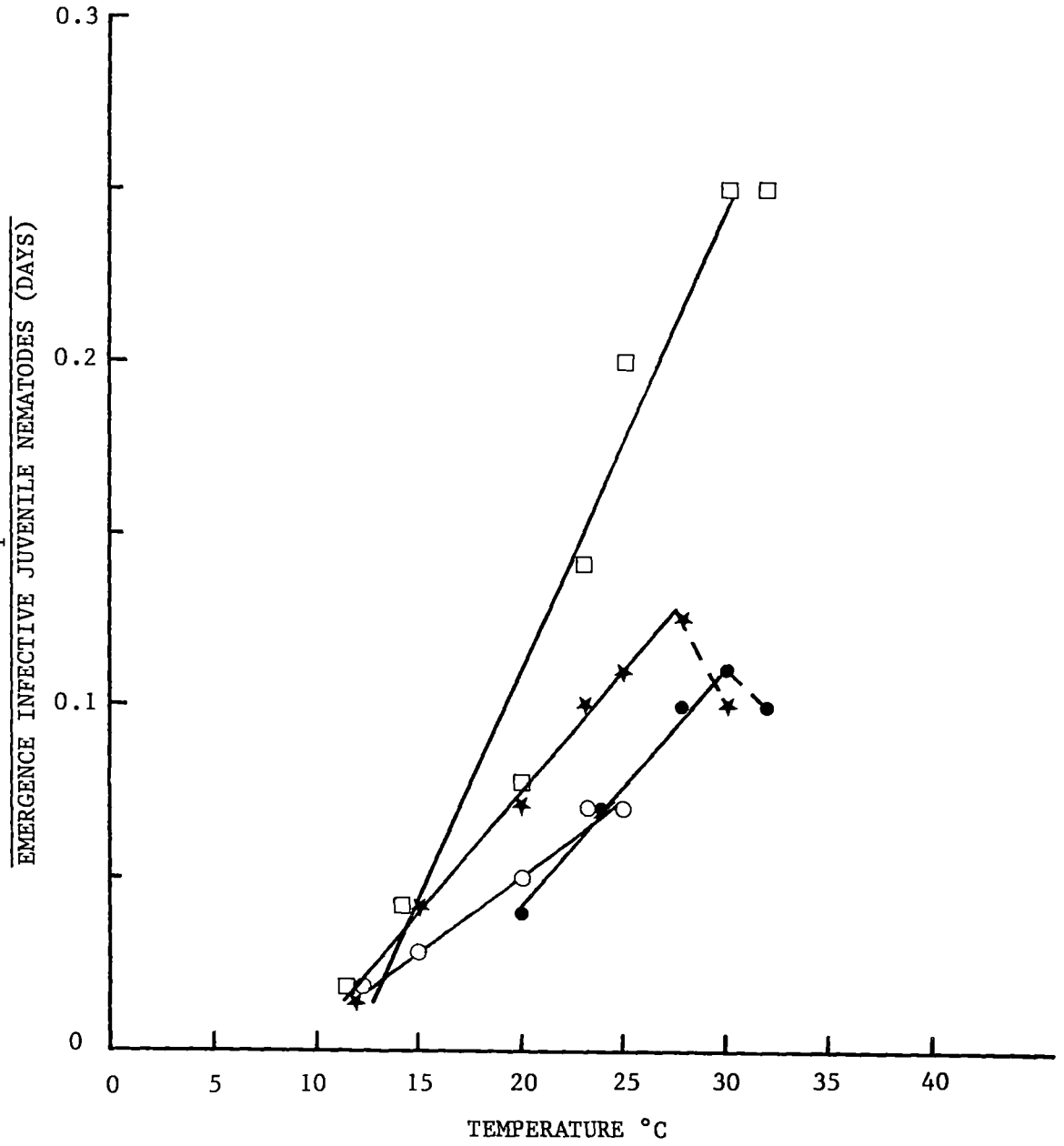
Key:

●——●	*Heterorhabditis* sp. D1,	Y = 0.007X - 0.093[a], r = 0.989[b], 13.5[c]
○——○	*Heterorhabditis heliothidis* T327	Y = 0.004X - 0.037[a], r = 0.989[b], 8.3[c]
✶——✶	*Steinernema feltiae* Agriotos	Y = 0.007X - 0.071[a], r = 0.996[b], 9.9[c]
□——□	*Steinernema glaseri* KG	Y = 0.013X - 0,153[a], r = 0.979[b], 11.8[c]

a Linear regression line

b Correlation coefficient

c Calculated developmental threshold temperature °C


0.3
0.2
0.1
0
1/EMERGENCE INFECTIVE JUVENILE NEMATODES (DAYS)
0
5
10
15
20
25
30
35
40
TEMPERATURE °C

strain infective juveniles emerged from 70% of infected *G. mellonella* larvae at 30°C but from 32° to 35°C there was only development to first generation adults.

S. glaseri KG strain was the only nematode not to be associated with the primary form of its bacterial symbiont. In comparison with *Heterorhabditis* sp. D1, *H. heliothidis* strain T327 and *S. feltiae* Agriotos strain, *S. glaseri* KG strain produced relatively few infective juveniles per cadaver (Fig. 17).

Figure 19 shows that *S. glaseri* has the greatest average rate of development whereas *S. feltiae* Agriotos strain is slightly faster than *Heterorhabditis* sp. D1 and *H. heliothidis* strain T327 has the lowest average rate of development. The calculated theoretical developmental threshold (zero) temperatures indicate that *S. feltiae* Agriotos strain and *H. heliothidis* strain T327 are better adapted for development at temperatures below 15°C and *Heterorhabditis* sp. D1 the least adapted for development at these temperatures.

vii) *Effect of Soil Type and Moisture on the Survival and Behaviour of Infective Juvenile Nematodes.*

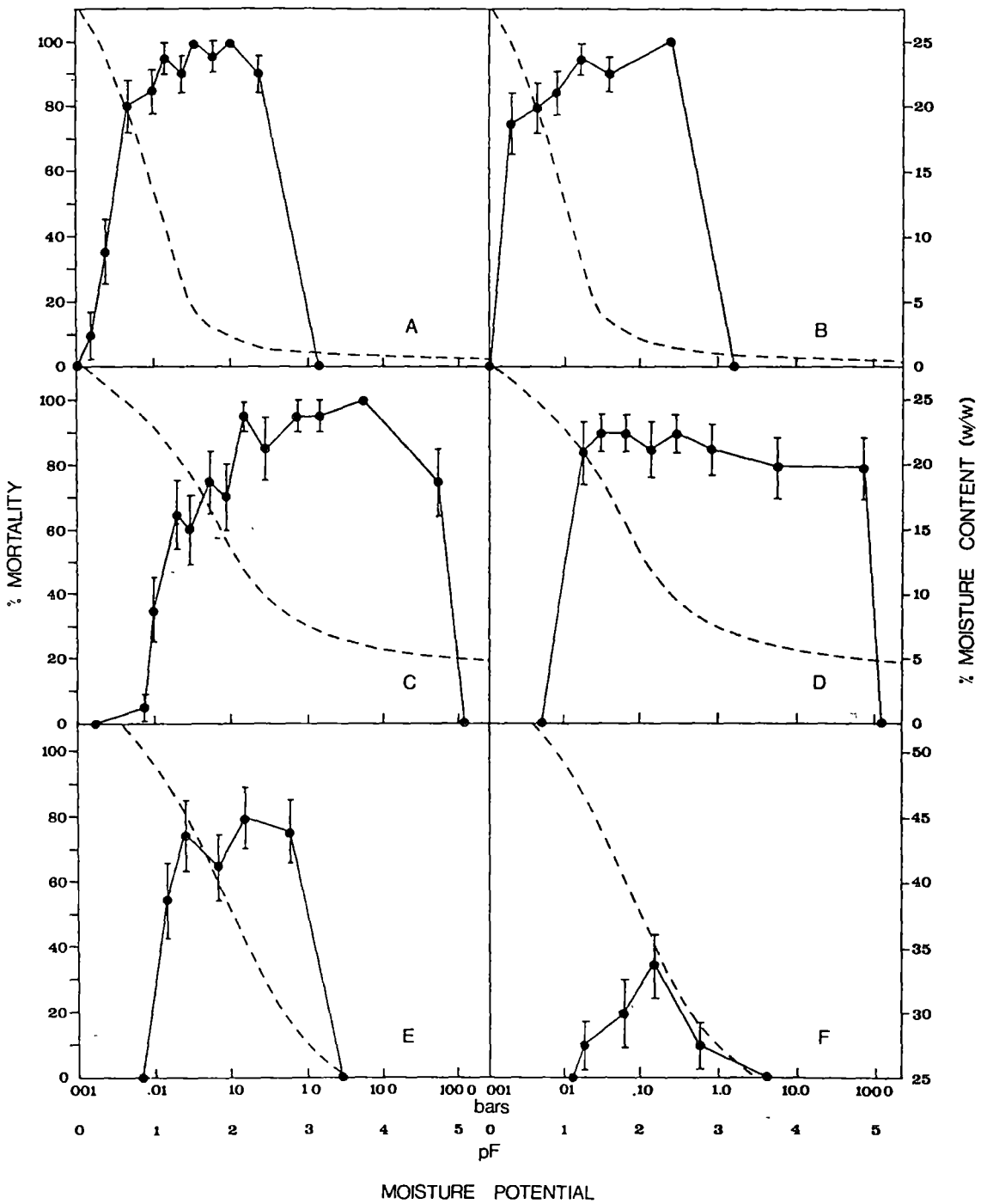
Figure 20 summarizes the results of effect of soil type and moisture on the survival and behaviour of infective juvenile nematodes and includes a moisture characteristic curve for each soil type. The soil analyses and classifications are given in Table IX. Both *Heterorhabditis* sp. D1 and *S. glaseri* KG strain infective juveniles parasitized *L. cuprina* larvae over a wide range of moisture potentials in different soil types. In the loamy sand, both nematodes parasitized *L. cuprina* larvae at moisture potentials equivalent to and below the permanent wilting point of plants.

TABLE IX

Soil classification and analysis.

Soil type	Soil composition (%) coarse sand	fine sand	silt	clay	organic carbon	pH (ratio 1:5)	Soil surface area ratio
Fine sand	25.0	65.0	7.0	<5.0	<0.5	5.50	1
Loamy sand	1.0	75.0	19.0	5.0	2.04	5.35	7
Sandy clay loam (Krasnozem)	6.0	23	19.0	52.0	0.8	5.50	396

Fig. 20 Mortality of post-feeding third instar *Lucilia cuprina* strain AM-3 larvae in three soil types of various moisture contents after individual exposure to dosages of either 10^3 *Heterorhabditis* sp. D1 (A, C, E) or *Steinernema glaseri* KG strain (B, D, F) infective juvenile nematodes in 80 g of soil (sand [A, B], loamy sand [C, D] and Krasnozem [E, F]) at 28°C. Each point shows per cent mortality for 20 *L. cuprina* larvae. The discontinuous line represents the moisture characteristic curve for each soil type and the vertical lines represent standard errors.


% MORTALITY
% MOISTURE CONTENT (w/w)
A
B
C
D
E
F
bars
pF
MOISTURE POTENTIAL

DISCUSSION

As for many phyto-parasitic and zoo-parasitic nematodes the soil environment is the natural reservoir for the free-living, non-feeding *Heterorhabditis* and *Steinernema* infective juvenile nematodes. The influence of various environmental factors, especially soil type, moisture and temperature, on the behaviour and survival of entomophagous nematodes are of particular importance during this period of time outside the insect host.

In this study a number of closely related nematode species/strains from different sources (Table 1) having quite widely different environmental conditions were compared.

Effect of Temperature on the Infectivity of the Infective Juvenile Nematode

The effect of temperature on any poikilothermic animal is of obvious importance and on very small animals such as nematodes, the effect of any change is almost immediate. Over that part of the temperature range in which a nematode remains viable, a Q_{10} type of relationship may be expected to occur; towards the lower extreme quiescence will occur and at higher extremes varying degrees of thermal damage will take place.

The majority of steinernematid nematodes were active at lower temperatures than were the heterorhabditids (Table III). In particular, infective juveniles of *S. kraussei* and the T335 strain of *S. bibionis* were mobile at 4°C and *S. bibionis* strain N60 at 3°C; the latter strain even responded to mechanical stimulation at 1°C. The low temperature immobilization of *S. feltiae* Agriotos strain (5°C) was comparable with

S. feltiae strain DD136 (Schmiege 1963). In contrast, none of the heterorhabditids tested were mobile at temperatures below 7°C.

The lower temperature limits for nematode movement of the Steinernematidae generally reflect the colder climates of their European and North American origins. *S. kraussei* and *S. feltiae* Agriotos strain originated from Poland and the U.S.S.R. respectively, while *S. glaseri* strain KG was originally isolated from North Carolina, U.S.A. Although both strains of *S. bibionis* were isolated from milder temperate areas of Australia, they were still mobile at 4°C. According to Bedding (pers. comm.) it is very likely that *S. bibionis* has been introduced accidentally into Australia, most probably from Europe. In contrast, all but one of the heterorhabditids were originally isolated from either the warm temperate areas of New Zealand or the temperate and tropical areas of Australia. *Heterorhabditis* spp. D1 and Q380 from tropical and subtropical areas of Australia respectively, would not normally have experienced temperatures below 10°-15°C in the field. Furthermore, their inability to move at 10°C and 14°C respectively, suggests that they have not been introduced into Australia from Europe or North America. The immobility of *H. heliothidis* strain NC, isolated from North Carolina, occurred at exactly the same temperature (7°C) as that for *S. glaseri* strain KG infective juveniles indicating that there is probably no intrinsic difference in this respect between *Heterorhabditis* and *Steinernema* species. This climatic influence has been retained even after rearing many generations of these nematodes in the laboratory at constant temperatures. This suggests that instead of temperature acclimitization, the nematode species/strains, through the process of evolution, have been genetically selected to survive the temperature ranges characteristic of their respective geographical regions.

In general, both the heterorhabditids and steinernematids parasitized insects over a similar range of temperatures with the majority of heterorhabditids favouring the warmer end of the temperature regime (Fig. 7). *S. glaseri* strain KG parasitized *L. cuprina* larvae between 8°C and 35°C while *S. feltiae* Agriotos strain parasitized *L. cuprina* larvae between 8°C and 32°C and both strains of *S. bibionis* parasitized between 4°C and 30°C. In contrast, *Heterorhabditis* sp. Q380 parasitized between 18°C and 35°C and *H. bacteriophora* parasitized between 10°C and 35°C.

Optimal infectivity of several steinernematids for *L. cuprina* larvae was maintained throughout most of the temperature range while the heterorhabditids appeared to have a more restricted temperature range for optimal infectivity. For example, *S. bibionis* strain T335 displayed optimal infectivity between 6°C and 25°C, within its temperature range for parasitization of 4°C to 30°C. In contrast, *H. heliothidis* strain NZ parasitized *L. cuprina* larvae between 10°C and 35°C with a distinct optimum at 20°C.

The temperature range of infectivity for insects also differed between nematodes of the same genus and isolated from the same geographical area. *Steinernema* sp. WI parasitized *L. cuprina* larvae only between 15°C and 20°C while *Steinernema* sp. WII parasitized *L. cuprina* larvae from 8°C to 28°C.

The results of the present study also demonstrate a strong interaction between nematode activity and the susceptibility of *L. cuprina* at various temperatures (Figs. 9-11, Tables IV and V). Within the temperature range 18°C to 28°C there was considerable variability in infectivity for *L. cuprina* larvae, even between strains of the same species

(e.g. *H. heliothidis* and *S. bibionis*). This variability can be partly explained in terms of the pupariation time of *L. cuprina* larvae at different temperatures (Table VI). At temperatures below 28°C, the pupariation time of *L. cuprina* larvae increased substantially, resulting in the prepuparia remaining susceptible to nematode infection for longer periods. Unlike lepidopteran pupae (Kaya and Hara 1981; Moyle and Kaya 1981b), *L. cuprina* larvae become almost completely invulnerable to parasitization soon after pupariation. Some of the nematode species/strains (e.g. *Heterorhabditis* spp. D1 and Q380) were less infective for *L. cuprina* larvae at 18°C than at 28°C indicating that the temperature effect on nematode activity was greater than the effect of temperature on the susceptibility of *L. cuprina* to nematode infection.

The interaction between nematode activity and pupariation time of *L. cuprina* at various temperatures will not be peculiar to this host alone. Many of those insects that pupate in soil will be similarly influenced and other insects not pupating will be less active and thus may become more vulnerable to nematode parasitization.

Effect of Temperature and pH on Survival of the Infective Juvenile Nematode

The free-living, non-feeding infective juveniles of many phyto-parasitic and zoo-parasitic nematodes must rely on their stored food reserves for the energy required to locate and infect their definitive hosts. Generally, the rate of nematode activity varies with temperature so that temperature is a major factor influencing the rate at which food reserves are utilized and, in turn, the survival of the infective juvenile nematode.

In the absence of their insect hosts the steinernematids survived for longer periods of time at various temperatures than did the heterorhabditids (Figs. 12 and 13). Wallace (1966) hypothesis that once a phyto-parasitic nematode enters the soil it moves until a host plant is located. The poor survival of *Heterorhabditis* sp. D1 and *H. heliothidis* strain T327 at temperatures above 10°C is likely to be related to a similar behaviour of continual movement and rapid utilization of their lipid reserves; within two weeks at 23°C the heterorhabditid infective juveniles recovered from sand appeared very transparent and were lethargic in their movements. The decrease in lipid content of the plant parasite *Meloidogyne javanica* with increasing age corresponded to a decrease in two enzymes, estrase and acid phosphatase, both of which function in the hydrolysis of lipids and phospholipids (Van Gundy *et al.* 1967) and a similar phenomonen may have occurred in *Heterorhabditis* sp. D1 and *H. heliothidis* strain T327, as they became transparent in time.

S. feltiae Agriotos strain and *S. glaseri* strain KG, the two steinernematids examined, presumably retained their lipid reserves for longer periods than the two heterorhabditids tested. However, unlike *S. feltiae* Agriotos strain which survived for only 16 weeks at 28°C, *S. glaseri* strain KG infective juveniles survived in excess of 32 weeks at each temperature tested. This longer period of survival of *S. glaseri* strain KG may have been because they became quiescent. This quiescent state was characterized by distinct 'coiled' postures (Fig. 14), similar to those produced by the phyto-parasites *Aphelenchus compositae* and *Ditylenchus dipsaci* (Croll 1970). *S. glaseri* strain KG infective juveniles probably retained their lipid reserves for much longer periods than *S. feltiae* Agriotos strain. As for several phyto-parasitic

nematodes (Van Gundy 1965), *S. glaseri* strain KG infective juveniles became active almost immediately when mechanically stimulated or when in the presence of an insect host. Although *S. feltiae* Agriotos strain infective juveniles were not observed in a 'coiled' state of quiescence, Danilov (1978) found that after the first two to four days in the field, *S. feltiae* Agriotos strain stopped migrating in soil and became quiescent. Maybe with the *S. feltiae* Agriotos strain, periods of quiescence were alternated with periods of activity. Saunders and All (1982) reported that after three weeks under field conditions there was a rapid decline in the number of *S. feltiae* infective juveniles recovered from soil and also a similar decline in their infectivity for *G. mellonella* larvae.

The infectivity of heterorhabditid and steinernematid nematodes for *L. cuprina* larvae declined with time in a similar manner to their survival, as did their mobility through a modified Baermann sieve (Appendix C). Extraction of nematodes from the substrate using a Baermann sieve (Whitehead and Hemming 1965) depends upon nematode activity and has been previously used to assess the mobility of various nematode species (Bird and Wallace 1965). However, Thomason *et al.* (1964) using *M. javanica* hypothesised that infectivity was a more specific property than mobility. Because *L. cuprina* larvae are vulnerable to nematode infection for only a short time (only until pupariation has occurred), a measure of nematode infectivity is also a good measure of their activity in relation to age. It indirectly measures the time taken for the nematodes to reach and infect the insect.

Ensheathed third-stage larvae of the vertebrate parasite *Haemonchus contortus* survive appreciably longer than exsheathed larvae and their

rate of water loss is slower (Ellenby 1969). In contrast, the majority of *S. glaseri* strain KG infective juveniles readily exsheathed in sand without any noticeable effect on their survival and infectivity. The heterorhabditids also exsheathed but not to the same extent as *S. glaseri* strain KG. Bedding (pers. comm.) considers that *Heterorhabditis* infective juveniles die soon after exsheathment unless they enter an insect host. However, many heterorhabditids were found dead ensheathed as well as exsheathed indicating that exsheathment was not the only cause of death.

Heterorhabditis sp. D1 and *S. feltiae* Agriotos strain infective juveniles survived for a longer period of time in aerated water compared with infective juveniles kept in moist sand at the same temperature (Fig. 15). Furthermore, the nematodes' lipid depletion may have been slower, implying reduced nematode activity. Perhaps because of sensory habituation, the infective juveniles remained motionless or 'less active' in water than if kept in sand, even though the aeration of the water was moving them passively through the medium. The infectivity of *Heterorhabditis* sp. D1 infective juveniles for *L. cuprina* larvae also decreased with age of nematode, although the infectivity of *S. feltiae* Agriotos strain for *L. cuprina* larvae remained reasonably constant (Fig. 16). It would appear that infectivity and physiological age are directly correlated in *Heterorhabditis* sp. D1 whereas they are independent of one another in *S. feltiae*. This may represent a basic intrinsic difference between the two genera.

Survival of *Heterorhabditis* and *Steinernema* infective juveniles in sand generally increased as the temperature decreased. Optimal survival of *Heterorhabditis* sp. D1 occurred at 15°C and for *H. heliothidis* strain

T327 at 10°C. However, as previously mentioned, the tropical origin of *Heterorhabditis* sp. D1 (Darwin, Australia) has meant that under field conditions the infective juveniles would normally have experienced a temperature between 20°C to 33°C. Optimal survival of *H. heliothidis* strain T327, *S. feltiae* Agriotos strain and *S. glaseri* strain KG infective juveniles occurred at 10°C in sand. Although *H. heliothidis* strain T327 survived for slightly longer periods at 28°C than at 23°C there was no significant difference between the two heterorhabditids.

Because *Heterorhabditis* infective juveniles survived for only a short period of time in sand, the effect of pH on their survival was investigated using *Heterorhabditis* sp. D1. Within the pH range of 4.5 to 6.5 (the pH range normally encountered in soils under agricultural or pastoral conditions), the survival of *Heterorhabditis* sp. D1 infective juveniles did not appear to be affected by pH. This tolerance to a wide pH range is advantageous for the nematode because firstly, many insect hosts are found in very diverse habitats and secondly, the soil pH may change considerably between different localities due to varying amounts and types of fertilizers and pesticides applied to various agricultural and pastoral systems.

The results suggest that the differences in survival of these nematode species/strains tested at various temperatures is likely to be the result of their original geographic and climatic habitats. Those nematode species/strains from warm, humid tropical and sub-tropical areas are more likely to infect insects throughout the year so that long-term survival of infective juveniles is not necessary. However, for those species/strains from temperate and cold climates, long-term survival of infective juveniles during the winter months when insect hosts are likely to be scarce would be an obvious advantage.

Effect of Temperature on Nematode Development and Reproduction

Once inside the insect host, the infective juvenile nematode is buffered to some extent from the influence of many environmental factors and in particular soil moisture. However, temperature is still an important factor influencing the nematode's behaviour and survival even when it is inside the host cadaver.

The range of temperature allowing development and reproduction inside *L. cuprina* cadavers was different for each nematode species/strain tested (Fig. 8). Generally, the steinernematids were able to develop and reproduce over a wider temperature range than the heterorhabditids. In particular, *S. bibionis* strain T335 reproduced within the temperature range of 10° to 28°C and *S. bibionis* strain N60 between 8° and 25°C. In contrast, the greatest range of temperature allowing development and reproduction of a heterorhabditid was only 12°C to 25°C for *H. heliothidis* strain T327. Also, two of the steinernematid species tested, *S. bibionis* strain N60 and *S. kraussei*, were able to reproduce below 10°C whereas none of the heterorhabditids tested reproduced below 12°C. However, the upper temperature limit for development and reproduction for both heterorhabditids and steinernematids was 32°C.

Different strains of the same species were also found to have different temperature limits for development and reproduction. The upper and lower temperature limit for reproduction of *H. heliothidis* strain T327 was 12°C and 25°C respectively, whereas for strains NC and NZ they were 15°C and 28°C respectively. Also, as mentioned previously, *S. bibionis* strain N60 reproduced within the temperature range 8°C to 25°C whereas *S. bibionis* strain T335 reproduced between 10°C and 28°C (Fig. 8).

An important difference between the heterorhabditid and steinernematid nematodes was the limited production of infective juvenile nematodes by the latter. Although the steinernematids were able to reproduce over a wide range of temperatures, the production of infective juveniles occurred over a substantially smaller range. In contrast, however, the production of *Heterorhabditis* infective juveniles coincided with the range of temperatures allowing development and reproduction. Because *Heterorhabditis* spp. survive for only short periods of time in the absence of insect hosts, the ability to produce infective juveniles across a fairly wide range of temperature would help to ensure survival of the field population. The results also suggest that (particularly with the steinernematids), inoculation of the surrounding soil with newly formed infective juveniles is not a continuous process but occurs spasmodically according to the temperature.

Another important difference between heterorhabditid and steinernematid nematodes was that whereas the former were able to reproduce within *L. cuprina* cadavers following medium and low dosage exposure at 28°C, there was almost no reproduction of steinernematids at this temperature. However, at temperatures below 25°C, steinernematids were frequently able to reproduce. The failure of *Steinernema* spp. to reproduce effectively in *L. cuprina* cadavers at 28°C, like the failure of *Heterorhabditis* and *Steinernema* spp. when applied in heavy dosages at lower temperatures, was apparently due to the contamination of the cadaver by high populations of foreign micro-organisms. The introduction of a high inoculum of gut bacteria (as a result of nematode infection via the anus) can result in failure of the nematode's bacterial symbiont to dominate within the host haemocoel and results in little or

no nematode reproduction. Unlike *Steinernema* spp., *Heterorhabditis* infective juveniles are able to penetrate the intersegmental membranes of their insect hosts thereby avoiding the gut flora and reducing the risk of contamination (Bedding and Molyneux 1982) (see Section III). Temperature will affect the rate of growth of these micro-organisms which, in turn will affect the rate of oxygen depletion and this may prevent reproduction or production of infective juveniles occurring inside the cadaver.

In general, the range of temperature allowing development and reproduction inside *L. cuprina* and *G. mellonella* cadavers infected with *Heterorhabditis* or *Steinernema* species was similar (cf Figs. 8 and 17). However, of the four nematode species/strains tested in detail in *G. mellonella* cadavers, *S. glaseri* strain KG and *S. feltiae* Agriotos strain were able to develop and reproduce at higher temperatures inside *G. mellonella* cadavers. Putrefaction of *G. mellonella* larvae infected with *Heterorhabditis* or *Steinernema* spp. at high temperatures was not observed. This is presumably because the main routes of entry for the infective juveniles are through the spiracles and oral cavity and also because the hind-gut flora is much less prolific in *G. mellonella* than in *L. cuprina* larvae.

The temperature limits for development and reproduction of *S. feltiae* Agriotos strain in *G. mellonella* cadavers differed from those reported for *S. feltiae* strain DD136 (Kaya 1977) and also from the limits found by Pye and Burman (1978) for the same Agriotos strain. In the present study, *S. feltiae* Agriotos strain infective juveniles emerged from cadavers after 70 days at 12°C (Fig. 18) whereas Pye and Burman (1978) stated that the lower temperature limit for development of

S. feltiae Agriotos strain was between 13°C and 15°C. However, these workers left the cadavers at 13°C for only 30 days. In the present study, *S. feltiae* Agriotos strain was found to develop to the adult stage but not reproduce at temperatures above 30°C. A similar observation was made by Hackett and Poinar (1973). In contrast, *S. feltiae* strain DD136 did not reproduce at temperatures above 28°C (Kaya 1977). However, the optimal temperature for development and reproduction of *S. feltiae* Agriotos strain (23°C) was similar to that reported for *S. feltiae* strain DD136 (25°C) (Kaya 1977). The optimal temperature for development and reproduction of *S. glaseri* strain KG in *G. mellonella* cadavers was similar to that found by Jackson (1962), but he found that *S. glaseri* reproduced at higher temperatures in axenic liquid media. This difference in the upper temperature limit for development may be due, in part, to competition with the symbiotic bacteria for oxygen inside the cadaver. Of the four nematode species tested for development and reproduction at various temperatures in *G. mellonella* cadavers, *Heterorhabditis* sp. D1 reproduced over the smallest temperature range (20°C-32°C) and *S. glaseri* KG strain reproduced over the greatest temperature range (12°C-32°C) (Fig. 17).

The temperature range allowing development and reproduction of these two species, as was demonstrated for their infectivity, can be associated with the temperatures usually encountered in the localities from which they were originally isolated.

Substantial differences in the number of infective juveniles produced per *G. mellonella* cadaver were obtained between the four nematode species/strains tested at various temperatures (Fig. 17). The maximum number of infective juveniles produced by *S. feltiae* Agriotos strain

occurred over a very narrow temperature range (23°C to 25°C) whereas *S. glaseri* strain KG produced the maximum number between 23°C and 30°C. The relatively low numbers of *S. glaseri* strain KG infective juveniles produced per cadaver compared with *S. feltiae* Agriotos strain was undoubtedly due to the absence of the primary form of its bacterial symbiont because the primary form allows faster and significantly greater reproduction by the nematodes than the secondary form (Akhurst 1980). *Heterorhabditis* sp. D1 not only produced many more infective juveniles per cadaver, but produced maximal numbers from 23°C to 32°C. In contrast, *H. heliothidis* strain T327 produced the maximal number of infective juveniles only at 20°C. The rapid decline in production of infective juvniles at higher temperatures may be the result of a lack of nutrition and/or oxygen for nematode maturation as a result of competition with its own bacterial symbiont. Presumably, with increases in temperature, the bacterial symbiont of *Heterorhabditis* sp. D1 does not increase its growth rate as quickly as the bacterial symbionts characteristic of the other nematode species/strains tested. The rapid decline in production of infective juveniles at lower temperatures is more likely to be the result of a direct temperature effect on the nematode rather than on its bacterial symbiont.

The time taken between infection and death of host and emergence of infective juvniles from cadavers was different for each of the nematode species/strains tested (Fig. 18). *S. glaseri* strain KG usually developed the fastest at each temperature tested and on no occasion took the longest time between infection and emergence. At most temperatures, the heterorhabditids took longer to complete their life cycle (i.e. infection of host to emergence of infective juvenile nematodes) than did the

steinernematids, although at 12°C, the rate of development of *S. feltiae* Agriotos was slower than that of *H. heliothidis* strain T327. Towards the upper temperature limit for development the time taken to complete the life cycle increased slightly due to heat stress and possible thermal damage to the nematodes. The rate of development of *S. feltiae* Agriotos strain was greatest between 25°C and 30°C (Fig. 19) which is comparable with that found by Reed and Carne (1967) for *S. feltiae* strain DD136. Similar rates of development of *S. feltiae* Agriotos strain at various temperatures were also obtained by Pye and Burman (1978) by measuring the time taken for infective juveniles to develop and reproduce.

The relationship between temperature and duration of development approximates to a hyperbola, so that if the reciprocal of developmental time (which may to taken as a measure of developmental rate) is plotted against temperature, an approximation to a straight line is obtained (Bursell 1974). If this line is extrapolated, the point at which it cuts the x-axis is the theoretical threshold temperature for development (developmental zero), i.e. it is the temperature at which no development will take place. This procedure has been used widely in studying the effects of temperature on the development of insects (Wigglesworth 1972) but does not appear to have been applied in the study of nematode development, and in particular, to the insect pathogenic nematodes *Heterorhabditis* and *Steinernema*. In practice, the theoretical calculation does not in fact provide the true threshold of development, for the temperature at which development ceases lies appreciably lower. However, in the present study, the theoretical threshold temperatures for nematode development gave a close approximation to the observed lower temperature limit for development (Fig. 19).

In all nematode species/strains tested in this study, the temperature range of growth for *Xenorhabdus* spp. was found to be broader than that of its nematode vector. Furthermore, most of the temperature ranges supporting only bacterial growth was at the highest temperatures and therefore of little relevance to nematode propagation. This supports the previous observations made by Milstead (1981) involving *H. bacteriophora*.

In contrast, the minimum temperature for reproduction of *Heterorhabditis* sp. D1 was 20°C whereas infective juveniles infected hosts and bacteria grew and killed hosts at 12°C. An advantage of this species/strain is that under fluctuating field temperatures, the cadaver would remain preserved, allowing nematode development and reproduction to resume when favourable conditions have been restored.

With all nematode species/strains tested in this study the range of temperature allowing parasitization of an insect was greater than the temperature range supporting nematode development and reproduction (Fig. 8). This is of little benefit to the nematode at the upper end of the temperature range because thermal damage soon resulted. However, at lower temperatures, nematodes will penetrate into the haemocoel and remain as infective juveniles. There they can remain without killing the host until temperatures rise allowing bacterial growth and nematode reproduction. In the meantime, the living insect may migrate and thus disseminate the nematode species/strain.

Effect of Soil Moisture on Nematode Infectivity

Nematodes are essentially aquatic animals requiring a moisture film in which to move whether it be in soil, plant tissue or animal hosts. Inside the protective environment of the cadaver, the behaviour and

survival of *Heterorhabditis* and *Steinernema* infective juveniles is not immediately influenced by changes in moisture levels of the external environment. However, once the infective juveniles have left the cadaver and entered into the soil environment, any changes in soil moisture will immediately influence their movement which, in turn, will influence their capacity to seek and infect various insect hosts.

In the present study, parasitization of *L. cuprina* larvae by *Heterorhabditis* sp. D1 and *S. glaseri* strain KG infective juveniles occurred across a similar range of moisture potentials for the same soil type with parasitization ceasing abruptly at both ends of the moisture regime (Fig. 20). However, the upper and lower moisture limits for nematode infection of *L. cuprina* larvae were found to vary between each soil type. In the loamy sand and Krasnozem, infection of *L. cuprina* larvae by *Heterorhabditis* sp. D1 and *S. glaseri* strain KG infective juveniles did not occur below a moisture potential of pF 1. In contrast, *L. cuprina* larvae were infected in sand by *Heterorhabditis* sp. D1 and *S. glaseri* strain KG infective juveniles until the sand was saturated with water (pF = 0). Peters (1953) and Wallace (1958a) found that nematode progression was slow in thick moisture films and between large particles where pores were full of water, since the external resistances perpendicular to the body axis are not sufficient to prevent lateral movemement. The infectivity of *L. cuprina* larvae in sand below a moisture potential of pF 1 might be explained in terms of soil structure. The soil structure was maintained at lower moisture potentials in sand than it was in the loamy sand and Krasnozem. The more uniform particle size distribution of the sand would presumably remain reasonably stable as the water content increased, until the decreasing cohesive forces

between the sand particles were overcome by the force of gravity. In contrast, the high percentage of silt and clay particles in both the loamy sand and Krasnozem tended to shift and pack more closely together as the moisture content of the soil increased, resulting in a substantial decrease in pore size. Presumably, this change in soil structure would restrict nematode movement more so in the loamy sand and Krasnozem than in the fine sand at equivalent low moisture potentials.

Infection of *L. cuprina* larvae would also become more difficult at low moisture potentials in much the same way as nematode movement became more difficult. When in a thick moisture film, the increase in lateral displacement of the nematode would not provide the nematode with sufficient purchase in which to push against the insect and gain entry through the cuticle and/or natural openings of the insect. Also, in very wet soils, *L. cuprina* larvae were found to be agitated and excessive insect movement would further make nematode entry difficult.

A further factor influencing nematode activity in soil at low moisture potentials is the availability of oxygen. The rates of diffusion of oxygen and carbon dioxide are related directly to the moisture content of the soil. Therefore, the nematode's ability to survive will depend on the length of time they are subjected to anaerobic conditions. Since aeration is not only a function of diffusion between soil aggregates but also through soil aggregates (Currie 1961; Russell 1973), the diffusion of gases through the Krasnozem and loamy sand would presumably take longer than through fine sand because of more tortuous pathways in the clay fraction. Also, Wallace (1962) demonstrated that the biological activity in a clay soil, as measured by oxygen consumption, was greater than in a sandy soil and so competition for oxygen

between nematodes and other organisms might have caused the inactivity of *Heterorhabditis* sp. D1 and *S. glaseri* strain KG in the loamy sand and Krasnozem before their inactivation in the fine sand. Although *S. feltiae* infective juveniles have been shown to survive for long periods in environments with a low oxygen level (Burman and Pye 1980a), the present study suggests that the low oxygen levels may interfere with the infection process.

At higher moisture potentials (lower negative values), *Heterorhabditis* sp. D1 and *S. glaseri* strain KG infective juveniles were able to parasitize *L. cuprina* larvae in the loamy sand at moisture potentials equivalent to or below the permanent wilting point of plants (pF 4.2). The finding of Bedding and Miller (1981a) that *S. bibionis* strain T335 was able to move in the absence of a moisture film may have some bearing on this observation. In contrast, Wallace (1960) found that the phytoparasite *Heterodera rostochiensis* could not migrate at moisture potentials in sandy loam or clay lower than pF 2.6 (although they could in peat).

Insect infection by nematodes which occurred below the wilting point of plants in the loamy soil might involve the interaction between surface area of the soil and its moisture content. Because of the high silt content in the loamy sand, its surface area was greater than the sand (7x) (Table IX) so that at equivalent moisture potentials above a particular value, a continuous moisture film would be maintained in the loamy sand but not in fine sand. This would enable the infective juveniles to move through the loamy sand, while in the fine sand, the nematodes would be held to the sand particles by the forces of surface tension. On the other hand, the surface area of the Krasnozem was greater than the loamy sand (70x) whereas the moisture content at equiv-

alent moisture potentials was only 4x as great. Therefore, at equivalent moisture potentials, the moisture film in the Krasnozem was much thinner than in the loamy sand and probably at pF 4.2 (the permanent wilting point of plants) too thin for nematode migration.

Since nematodes are apparently unable to move soil particles aside, their mobility depends upon the presence of existing channels of sufficient diameter (Wallace 1958a, 1961). The infectivity of *S. glaseri* strain KG for *L. cuprina* larvae in the Krasnozem was reduced markedly compared with its infectivity for *L. cuprina* in the loamy sand and fine sand and with the infectivity of *Heterorhabditis* sp. D1 for *L. cuprina* in the three soil types. The much lower level of infectivity by *S. glaseri* strain KG in the Krasnozem is likely to be because the infective juveniles of *S. glaseri* strain KG are too large (length 1-3 mm, width 45 µm) to move through the soil pores of this soil type whereas the relatively small *Heterorhabditis* sp. D1 infective juveniles (length 0.5 mm, width 24 µm) are not.

Many workers have studied the movement and infectivity of phytoparasitic nematodes in soil in relation to moisture potentials (Seinhorst 1950; Wallace 1961; Fidler and Bevan 1963; Kable and Mai 1968; Grandison 1973) but this is the first time moisture potentials have been related to the infectivity of entomopathogenic nematodes. Although Georgis and Poinar (1983) studied the influence of three different soil types on the movement of *S. feltiae* Breton strain, these were all examined at the same moisture content. Consequently they did not compare nematode movement in the different soil types at equivalent amounts of free water available for nematode movement in the soil. The influence of soil texture, structure, organic matter and clay mineralogy

on the soil moisture characteristic curve has now been successfully modelled (Williams *et al.* 1983), so that given a moisture characteristic curve, predictions may be made concerning the influence of these factors on the moisture potential. The use of predictive models utilizing the moisture characteristic curves of different soil types could become an important tool for predicting how *Heterorhabditis* and *Steinernema* will behave in a particular soil. This study is especially important now that low cost *in vitro* mass-production of these nematodes (Bedding 1981, 1983) allows their use over large areas of agricultural and pastoral land.

In conclusion, the results of this section of the present study have emphasized the importance of soil type, moisture and temperature on the behaviour and survival of the infective juvenile nematode. Not only does their original climatic environment influence their behaviour, but each nematode species/strain differs from the others in its response to temperature and this occurs even between strains of the same species and other species from the same geographical region. Therefore, each nematode species/strain must be tested to determine its 'climatic' range and suitability for each field situation. The temperature range for nematode development and reproduction also differs for each nematode species/strain tested and is influenced, in particular, by the number of infective juveniles entering insect hosts. Nematode dosage rates and size and type of insect host in relation to temperatures will need to be considered especially if a residual population of nematodes is to be established permanently in the field. The substantial differences in the number of infective juveniles produced per cadaver within that reproduction range is of particular importance in relation to the mass

production of these nematodes for large-scale useage. In order for these nematodes to become an effective alternative to insecticides, rearing and application costs must be kept to a minimum; therefore the temperature giving maximal numbers of infective juvenile per unit cost is of obvious importance.

SECTION III

TRANSITION FROM THE FREE-LIVING TO THE PARASITIC PHASE BY INFECTIVE *HETERORHABDITIS* JUVENILE NEMATODES

INTRODUCTION

Unlike the free-living, infective-stage juveniles of many insect parasitic nematodes (Christie 1936; Poinar and Doncaster 1965; Petersen and Chapman 1970; Bedding 1972) there is no record of rhabditid nematodes penetrating the external cuticle of insects. *Steinernema* infective-stage juveniles enter the insect host via mouth, anus and/or spiracles (Poinar and Himsworth 1967; Poinar 1979) without the aid of a stylet or glandular secretions. Penetration through the gut wall into the insect's haemocoel is reputedly due to mechanical pressure (Poinar and Himsworth 1967).

It has been assumed that *Heterorhabditis* infective-stage juveniles, like those of *Steinernema* are only able to enter their hosts via mouth, anus and spiracles (Poinar 1975; Khan *et al.* 1976; Wouts 1979). In this section, investigations are made concerning the mode of entry of *Heterorhabditis* infective-stage juveniles into a number of different insect hosts using light and scanning electron microscopy (SEM).

MATERIALS AND METHODS

i) *Light Microscopy*

Infective-stage juveniles of *Heterorhabditis* sp. D1, *H. bacteriophora* and *H. heliothidis* strain T327 were added to second instar *L. cuprina* larvae and early instar *G. mellonella* larvae and *O. sulcatus* larvae in 1.5% purified agar. The insect larvae were first inactivated by immersion in water at 60°C for 1-3 min. (depending on size of insect) to prevent their migration from the agar. Each block of agar containing an insect was suspended from a coverslip and nematodes transferred to the agar/coverslip interface by means of a fine hair. Specimens were examined immediately using a Zeiss light microscope at 400x.

ii) *Scanning Electron Microscopy*

a) *Insect preparation* - Third instar larvae and prepuparia of *L. cuprina* exposed to *Heterorhabditis* infection in sand overnight at 23°C were quickly inactivated by immersion in water at 60°C, washed several times in insect Ringer's solution and briefly washed in distilled water. Specimens were then fixed by immersion in Carnoys fixative (Norris and Upton 1974) for 2h at 23°C, washed once in distilled water and dehydrated for 1h in 30% and 50% ethanol, 16h in 70% ethanol, 1h in 80%, 90% and 95% ethanol and 2 x 1h in fresh absolute alcohol.

b) *Nematode preparation* - Exsheathed *Heterorhabditis* and *Steinernema* infective-stage juveniles were obtained by dissecting third instar *L. cuprina* larvae that had been exposed to nematode infection in sand overnight at 20°C. Infective juveniles were washed several times in insect Ringer's solution followed by a brief wash in distilled water. They were then transferred using a fine hair and fixed by immersion in a mixture of 5% glutaraldehyde and 4% paraformaldehyde in 0.1M Na-cacodylate buffer at pH 7.3 for 2h at 23°C. Specimens were then washed in several changes of buffer and post-fixed for 1h at 23°C in 1% osmium tetroxide in the same buffer. Specimens were again washed in several changes of buffer followed by a brief wash in distilled water. Specimens were dehydrated for 1h in 30% and 50% ethanol, 16h in 70% ethanol, 1h in 80%, 90% and 95% ethanol and 3 x 45 min. in fresh absolute alcohol.

c) *Examination of specimens* - All insect and nematode preparations were air dried and mounted onto brass SEM (1.0 cm diameter) stubs using double sided adhesive tape (Scotch, Australia Pty. Ltd.).

Silver conductive paint was applied to ensure contact between the specimen and upper surfaces of stub. Insect and infective-stage juvenile preparations were coated with gold at 300Å in a Joel Sputter JFC-1100 and examined with a Philips 505 scanning electron microscope.

OBSERVATIONS AND RESULTS

i) *Penetration*

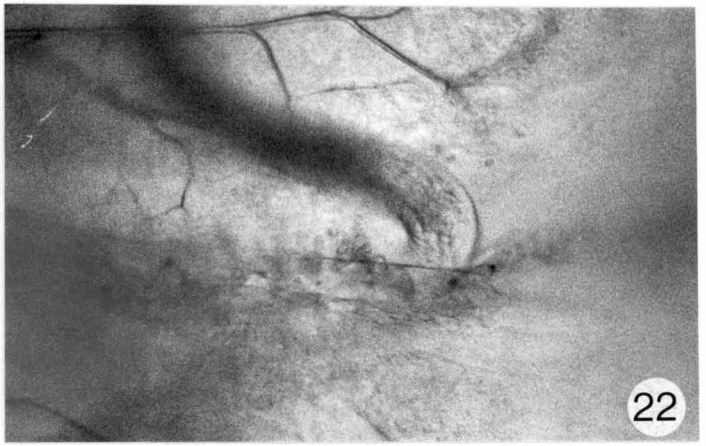
Heterorhabditis infective juveniles moved over the cuticle of insects for several minutes to several hours before attempting penetration. The nematodes usually kept close to the insect's surface moving forwards, and occasionally backwards, with the head end exploring crevices and folds of the insect's cuticle. During this exploratory period, about 25-33% of the infective juveniles shed the enclosing L2 cuticle.

Cuticular penetration of *O. sulcatus* and *L. cuprina* larvae and *Mastotermes darwiniensis* Froggatt nymphs (Bedding, pers. comm.) was observed on many occasions. It was usually preceded by the nematode forcing its head end into a cuticular fold (Fig. 22), or, in the case of *M. darwiniensis*, often between the leg joints (Fig. 21). However, it also occurred on areas of flat cuticle (Fig. 23) where the cuticle was flexible enough to allow the nematode to readily depress the surface. Although infective juveniles on *G. mellonella* larvae were often seen entering the spiracles, they were seldom observed penetrating the cuticle. However, cuticular penetration was observed between the crochets on the prolegs of *G. mellonella* larvae.

Following probing movements with the head end, the body of the infective juvenile became immobile except for the head end which moved

Figs. 21-24 Infective juvenile nematodes of *Heterorhabditis* sp. Dl. 21: (220x) Penetrating intersegmental membrane at a leg joint of *Mastotermes darwiniensis* (courtesy of Dr. R.A. Bedding). 22: (370x) Probing at intersegmental membrane of post-feeding, third instar *Lucilia cuprina* strain AM-3 larva. 23: (330x) Exsheathed infective juvenile nematode penetrating and ensheathed infective juvenile exploring cuticle of first instar *L. cuprina* strain AM-3 larva. 24: (170x) Empty sheaths protruding from anal region of post-feeding third instar *L. cuprina* strain AM-3 larva following entry of infective juvenile nematodes.


21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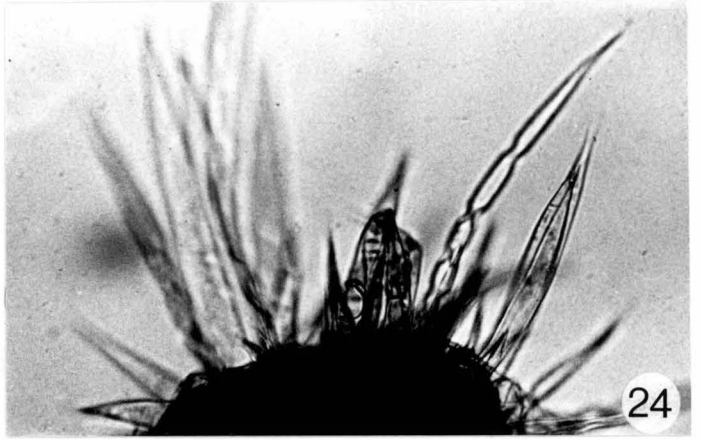
23

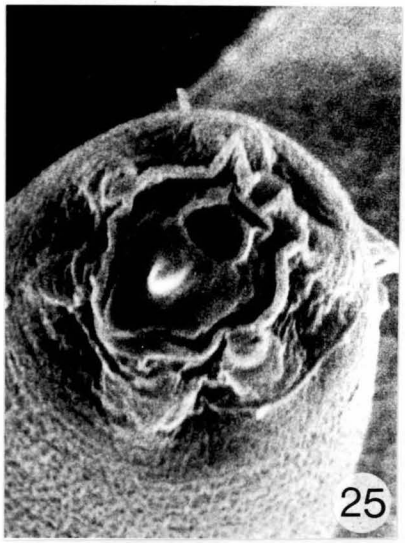

24

repetitively in a dorso-ventrally directed arc so that the terminal tooth (Figs. 25 and 26) abraded or scratched the insect's cuticle. These movements occurred at about one every second at 20°C and continued until the insect's cuticle ruptured, which occurred after approximately 30 mins. While many infectives had exsheathed before commencing to enter, others started this process prior to exsheathment; in the latter case, the dorsal tooth also aided rupture of the nematode's sheath.

No nematode glandular secretions, such as occur during penetration of *Tripius* (Poinar and Doncaster 1965) were observed. However, sheaths of some nematodes that penetrated remained adhering to the entry sites (Figs. 24 and 28) possibly through the presence of such secretions or of insect haemolymph.

Once rupture of the insect's cuticle had been effected, penetration by the nematode was usually rapid, often within a few minutes. In most instances, the head and oesophageal region entered rapidly, followed by a pause of a minute or more during exploratory movements inside the host; the nematode then penetrated distances of 20 to 100 µm at a time followed by pauses during which it often backed out a little before penetrating further. Frequently, once one infective had broken the cuticle, others entered through the same wound. Where very large numbers of infectives were present, the insect larva was sometimes severely ruptured allowing substantial leakage of host haemolymph into the surrounding medium. Indeed, where *O. sulcatus* larvae were placed in small vials of sand with hundreds of infectives, the sand became blackened with melanised insect haemolymph. In comparison, *Steinernema* infective juveniles did not rupture the cuticle of *O. sulcatus* larvae and were found only in the anal and oral cavities.

Figs. 25-28 Scanning electron micrographs (S.E.M.) of anterior end of exsheathed *Heterorhabditis* sp. D1 infective juvenile nematode. 25: (5,200x) *En-face* view, showing terminal tooth and basal plate. 26: (8,900x) Lateral view showing terminal tooth. 27: (3,000x) S.E.M. of anterior end of exsheathed *Steinernema glaseri* KG strain infective juvenile nematode. 28: (350x) S.E.M. showing empty sheaths left after penetration of post-feeding third instar *Lucilia cuprina* strain AM-3 larva by *Heterorhabditis* sp. D1 infective juvenile nematodes.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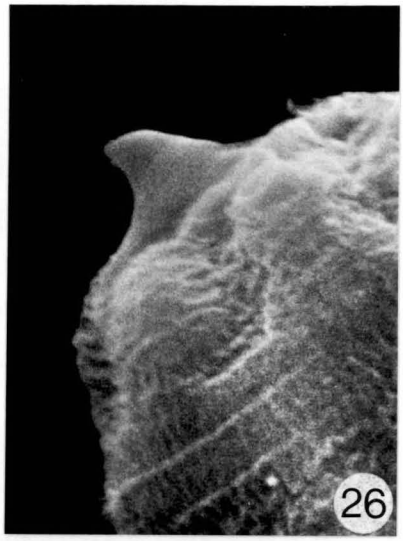
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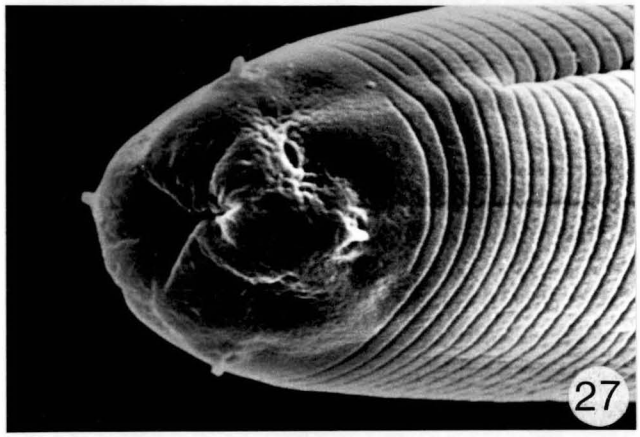
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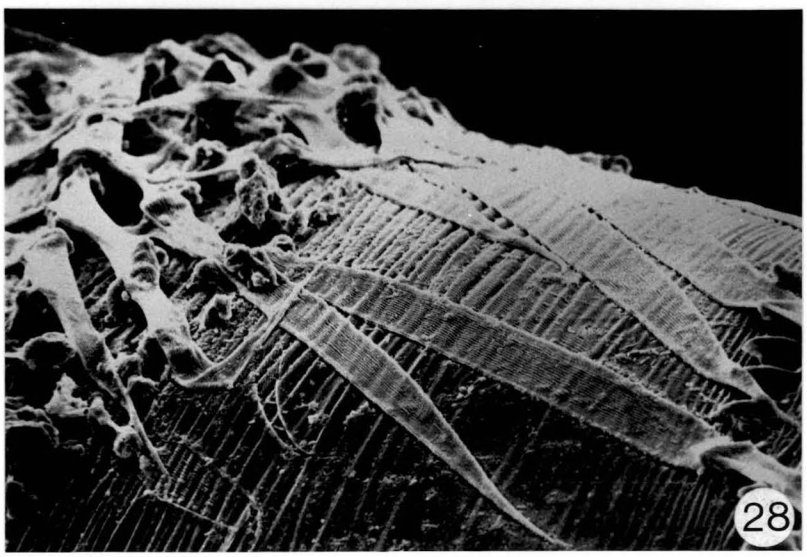
28

Fig. 29 (400x) Scanning electron micrograph showing *Heterorhabditis* sp. D1 infective juvenile nematodes penetrating a post-feeding third instar *Lucilia cuprina* strain AM-3 larva via the anus.

In addition to cuticular penetration, *Heterorhabditis* infective juveniles were also observed entering their insect hosts via the oral, anal and spiracular openings (Fig. 29).

ii) *The Anterior Tooth*

The anterior tooth of infective *Heterorhabditis* sp. Dl is only readily apparent after exsheathment. It is similar in *H. bacteriophora* and *H. heliothidis* being concave ventrally and convex dorsally so that its sharp point is directed ventrally (Fig. 26) in the direction in which it is used. It is inserted on an apparently rigid basal plate (Fig. 25) and light microscopy indicates that both tooth and basal plate are strongly sclerotised.

Examination of exsheathed infective juveniles of *S. glaseri* (Fig. 27), *S. feltiae* Agriotos strain and *S. bibionis* strain T335 showed that there was no tooth present in these nematodes.

DISCUSSION

Both *Heterorhabditis* and *Steinernema* infective juveniles enter insects via mouth, anus and/or spiracles (Poinar 1979) and in addition to must penetrate the cuticle of the alimentary tract or tracheae of the host in order to reach the insect's haemocoel. The high internal hydrostatic pressure (characteristic of nematodes), combined with the minute diameter of the infective juvenile's head (ca 8-15 μm) doubtless enables it to push through thin internal cuticle. However, this alone is apparently inadequate to effect penetration of the tougher outer cuticle of insects. However, the possession of a terminal tooth (Figs. 25 and 26) by *Heterorhabditis* infective juveniles enables them to penetrate the external cuticle of insects thus giving heterorhabditids an additional route of entry (Bedding and Molyneux 1982).

Advantages of cuticular penetration are not just that it provides an additional route of entry into any insect but maybe is the only route of entry into some insects. A number of insects have modified, protected or very minute spiracles, and/or mouth and anus may be too small or too tightly closed for nematode entry. This is particularly true of various endopterygote pupae. Penetration of the outer cuticle has an additional advantage over penetration via the hindgut because in the latter situation, bacterial flora may be carried into the insect's haemocoel. In parasitization of blowfly larvae by *Steinernema* species, this introduction of hindgut bacteria may result in the failure of the nematode's bacterial symbiont to dominate within the host haemocoel and consequently there is little or no nematode reproduction (Molyneux *et al.* 1983). Poinar (1979) showed that bacteria may be carried on the outer cuticle of infective juvenile nematodes. Since in *Heterorhabditis* species, the cuticle is shed just before or during penetration (Figs. 24 and 28), the infective juvenile can enter with a surface that is largely free of bacteria. The few foreign bacteria introduced during penetration are likely to be destroyed by the insect's defence mechanisms (Götz *et al.* 1981). Consequently, the infective juveniles release symbiotic bacteria into an essentially aseptic haemocoel which ensures that the symbiont dominates the bacterial flora long after the insect dies.

SECTION IV

THE INFECTIVITY OF DIFFERENT *HETERORHABDITIS* AND *STEINERNEMA* SPECIES/STRAINS FOR VARIOUS INSECT HOSTS

INTRODUCTION

Heterorhabditid and steinernematid nematodes are obligate pathogens of insects and numerous examples of insect parasitization by these nematodes have been demonstrated (Poinar 1979). The host range of at least some of these nematodes has been found to be very wide (Dutky 1974; Laumond *et al.* 1979; Poinar 1979) but most infectivity studies have been conducted under highly artificial laboratory conditions. Furthermore, little effort has been made to quantify the infectivity of different nematode species/strains for various insects.

Most infectivity studies have used strains of *S. feltiae* against a number of different insects on moist filter paper (Poinar 1979; Gaugler 1981) and the inconsistent results obtained using *S. feltiae* against foliage-feeding insects in the field have been responsible for a decline in the interest in the use of these nematodes as biocontrol agents (Gaugler 1981).

However, a large number of endopterygote insects live in the soil or, after cessation of feeding, enter the soil to pupate. It is in this situation where relative humidity is near 100% and insect larvae are surrounded by soil that nematode infection may most readily occur (Bedding and Akhurst 1975).

In this section, an attempt has been made to approximate more closely the conditions under which many soil-dwelling insects would normally be parasitized by these nematodes in the field. At the same time, a uniform and easily replicated method of testing was devised in order to compare the infectivity of several nematode species/strains for a number of different insects that live in, or pupate in soil.

MATERIALS AND METHODS

Final instar insects were exposed individually to nematodes within specimen jars filled with sand of moisture content ca. 7% (pF = 1.3). For insect species that normally pupate in soil, larvae were placed on the sand surface after the addition of the nematodes and the lids were screwed on after the larvae had started to burrow. *G. mellonella* larvae, which pupate above ground, were placed in the containers before the sand and nematodes were added.

The dosage range* used for any nematode/insect combination was chosen following preliminary experiments using one or two dosages. There were 20 replications of each dosage for each nematode species/strain. For each insect species there were 40 nematode-free controls. After 14 days incubation at a temperature considered to be appropriate for the insect (18°C for *A. couloni, G. mellonella, O. sulcatus,* and *S. humeralis,* or 28°C for *C. vicina, C. stygia, L. cuprina, L. sericata,* and *H. punctiger*), the insects were removed from the sand, dissected in insect Ringer's solution and microscopically examined for nematode parasitization.

It was not feasible to examine simultaneously the full spectrum of nematode species/strains with each insect species or the complete range of insects. The nematode species/strains were divided into two groups for each insect species; when each group of nematode species/strains was tested, the D1 strain of *Heterorhabditis* was included as a standard as well as 20 nematode-free controls.

LD_{50} and LD_{90} values for each nematode/insect combination were computed using probit analysis (Finney 1971). The reproducibility of results using this method was checked using *O. sulcatus* collected from a

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* Nematode dosage range was:
10^0, 10^1, 10^2, 10^3, 10^4 for *L. cuprina, L. sericata, C. vicina, C. stygia, A. couloni*;
10^0, $10^{0.5}$, 10^1, $10^{1.5}$, 10^2, $10^{2.5}$, 10^3, $10^{3.5}$, 10^4 for *O. sulcatus, S. humeralis*;
2^0, 2^1, 2^2, 2^3, 2^4, 2^5 for *H. punctiger, G. mellonella.*

different source several months later and nematodes (three species/strains) harvested from cultures at this time. The coincidence of the two probit lines obtained for each of these three species/strains was tested using the general linear model concept in the GENSTAT statistical package (Alvey *et al.* 1977). The reproducibility of results was also checked using *Heterorhabditis* sp. D1 against *L. cuprina* on 14 separate occasions, against *C. vicina* on three occasions and against *H. punctiger* on two occasions.

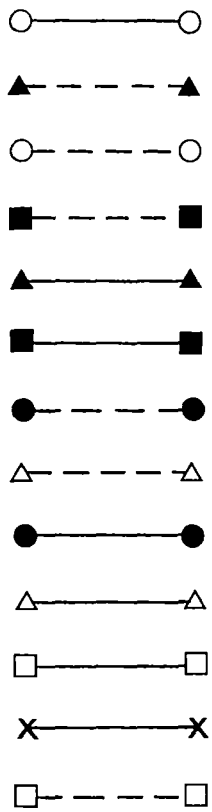
RESULTS

The results of dosage mortality experiments are summarised in Figures 30-33, and Table X. There was no significant difference between the results obtained for any nematode/insect combination repeated on different occasions. LD_{50} and LD_{90} values were not calculated for *G. mellonella* larvae because there were too few data points below 100%. Similarly values were not calculated for some other nematode/insect combinations (Table X) where mortality did not reach 50% at any dosage.

In no case did *Steinernema* spp. reproduce effectively at any dosage in blowfly cadavers (*C. vicina, C. stygia, L. cuprina* and *L. sericata*) at 28°C and the cadavers were invariably fetid. Occasionally at dosages of 10^1 and 10^2 infective juveniles, some reproduction was observed in *C. vicina* and *C. stygia* cadavers, although infective juvenile nematodes were not produced. *Heterorhabditis* spp. reproduced in all blowfly cadavers resulting from dosages of 10^0, 10^1 and 10^2 except *H. heliothidis* strain T327 which only reproduced occasionally. Little reproduction occurred in cadavers following dosages of 10^3 and none from

Fig. 30 Dosage/mortality lines for final instar *Galleria mellonella* (A, B) and *Heliothis punctiger* (C, D) larvae exposed to heterorhabditid and steinernematid infective juvenile nematodes in 80 g sand (pF = 1.3) at 18° and 28°C, respectively (Heterorhabditids - A, C; Steinernematids - B, D).

Key:



Heterorhabditis sp. D1;

Heterorhabditis sp. V16;

Heterorhabditis heliothidis T327;

Heterorhabditis heliothidis NC;

Heterorhabditis heliothidis NZ;

Heterorhabditis bacteriophora;

Steinernema sp. Q393;

Steinernema bibionis N60;

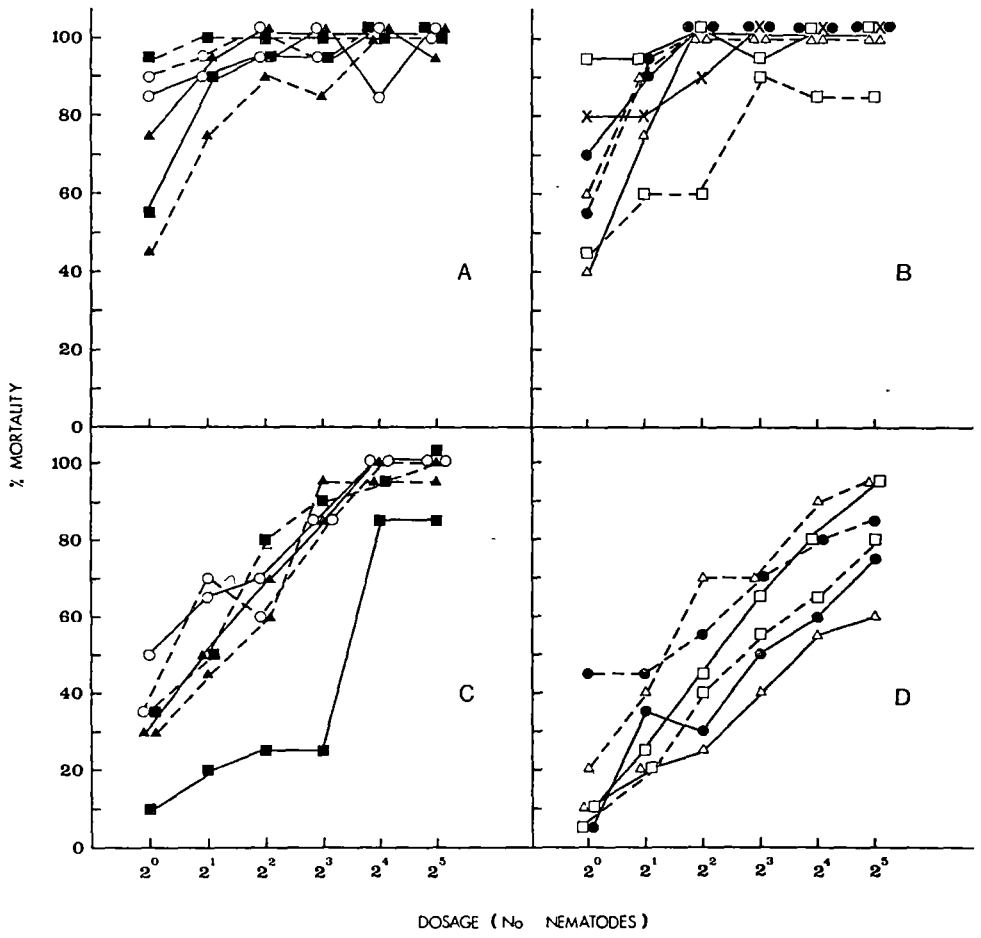
Steinernema bibionis T335;

Steinernema feltiae Agriotos;

Steinernema glaseri KG;

Steinernema kraussei.

Undescribed steinernematid Q1;


% MORTALITY
100
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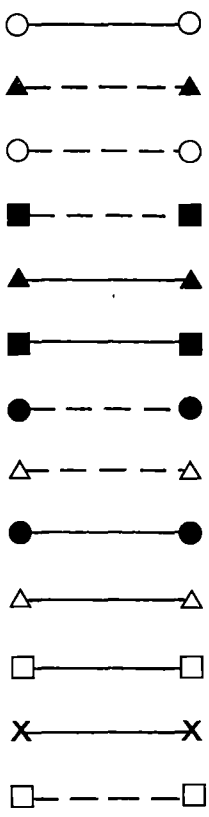
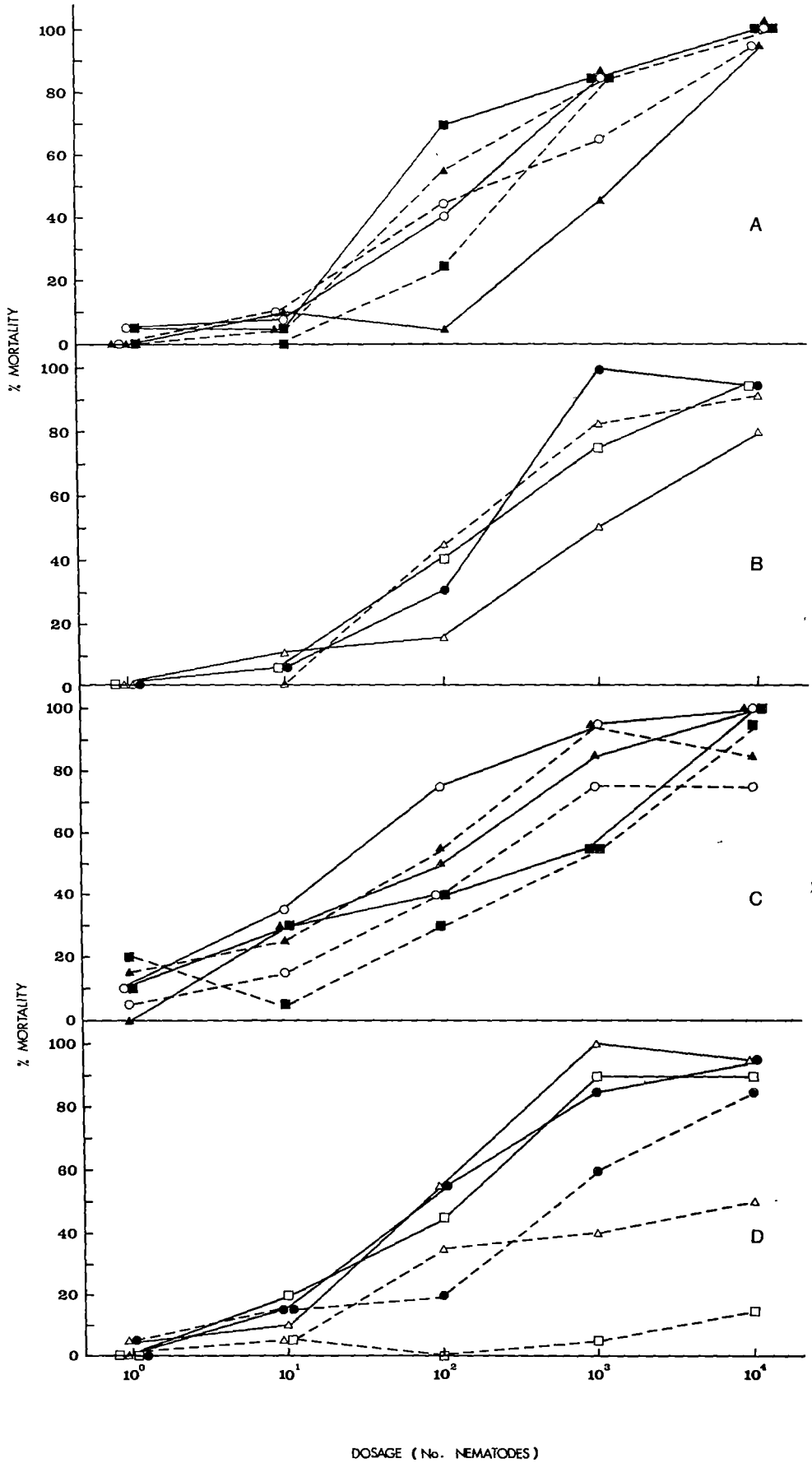
60
40
20
0
A
B
C
D
2^0 2^1 2^2 2^3 2^4 2^5
DOSAGE (No NEMATODES)

Fig. 31 Dosage/mortality lines for post-feeding third instar *Lucilia sericata* Perth strain (A, B) and *Lucilia cuprina* strain AM-3 (C, D) larvae exposed to heterorhabditid and steinernematid infective juvenile nematodes in 80 g sand (pF = 1.3) at 28°C (Heterorhabditids - A, C; Steinernematids - B, D).

Key:



Heterorhabditis sp. D1;
Heterorhabditis sp. V16;
Heterorhabditis heliothidis T327;
Heterorhabditis heliothidis NC;
Heterorhabditis heliothidis NZ;
Heterorhabditis bacteriophora;
Steinernema sp. Q393;
Steinernema bibionis N60;
Steinernema bibionis T335;
Steinernema feltiae Agriotos;
Steinernema glaseri KG;
Steinernema kraussei.
Undescribed steinernematid Q1;


% MORTALITY
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B
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10^0
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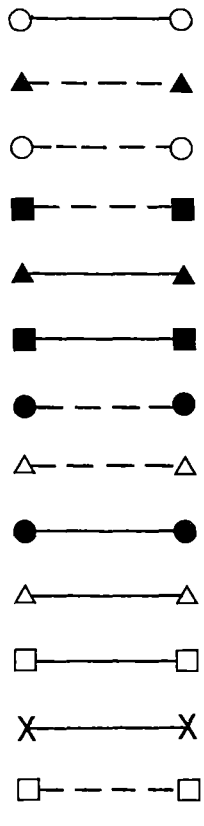
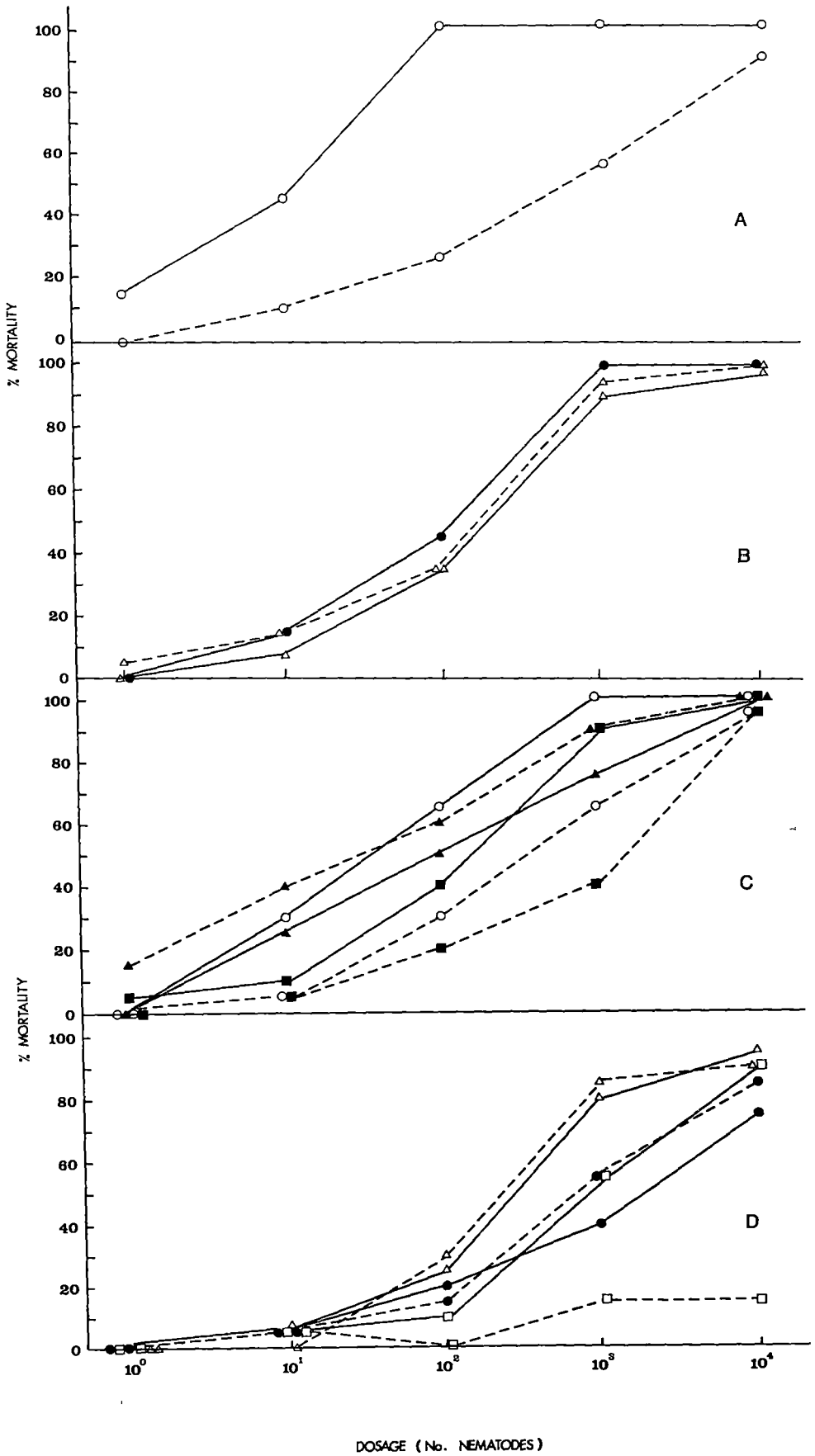
10^3
10^4
DOSAGE (No. NEMATODES)

Fig. 32 Dosage/mortality lines for post-feeding third instar *Calliphora stygia* (A, B) and *Calliphora vicina* Stowell strain (C, D) larvae exposed to heterorhabditid and steinernematid infective juvenile nematodes in 80 g sand (pF = 1.3) at 28°C. (Heterorhabditids - A, C; Steinernematids - B, D).

Key:



Heterorhabditis sp. D1;
Heterorhabditis sp. V16;
Heterorhabditis heliothidis T327;
Heterorhabditis heliothidis NC;
Heterorhabditis heliothidis NZ;
Heterorhabditis bacteriophora;
Steinernema sp. Q393;
Steinernema bibionis N60;
Steinernema bibionis T335;
Steinernema feltiae Agriotos;
Steinernema glaseri KG;
Steinernema kraussei.
Undescribed steinernematid Q1;


% MORTALITY
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A
B
C
D
10^0
10^1
10^2
10^3
10^4
DOSAGE (No. NEMATODES)

Fig. 33 Dosage/mortality lines for final instar *Otiorhynchus sulcatus* (A), *Sitona humeralis* (B) and *Adoryphorus couloni* (C, D) larvae exposed to heterorhabditid and steinernematid infective juvenile nematodes in 80 g sand (pF = 1.3) at 18°C (Heterorhabditids - A, B, C; Steinernematids - A, B, C, D).

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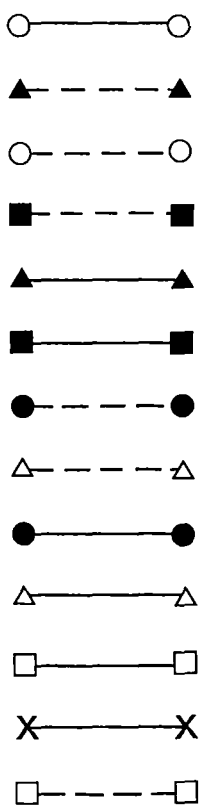
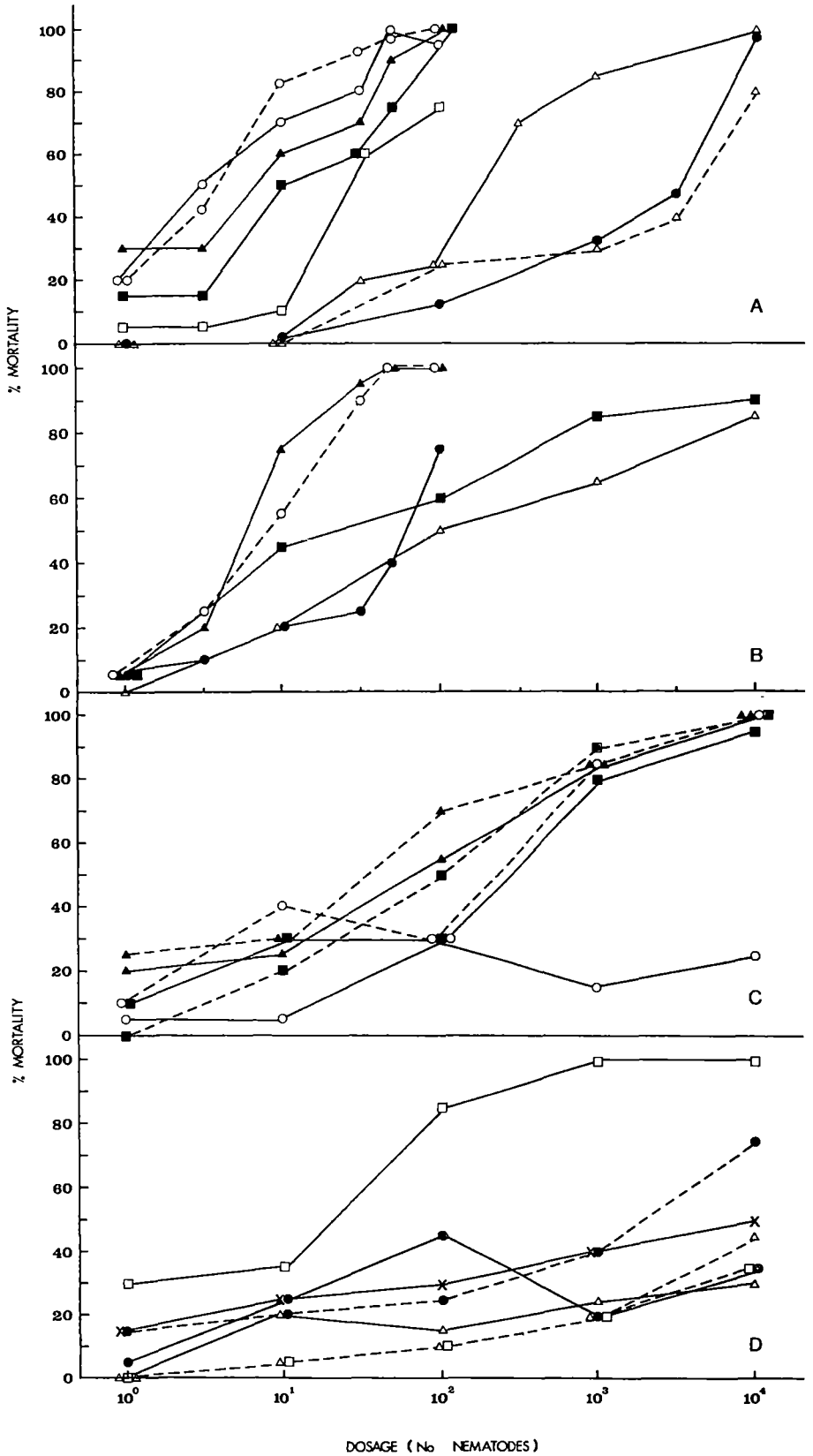


Heterorhabditis sp. D1;
Heterorhabditis sp. V16;
Heterorhabditis heliothidis T327;
Heterorhabditis heliothidis NC;
Heterorhabditis heliothidis NZ;
Heterorhabditis bacteriophora;
Steinernema sp. Q393;
Steinernema bibionis N60;
Steinernema bibionis T335;
Steinernema feltiae Agriotos;
Steinernema glaseri KG;
Steinernema kraussei.
Undescribed steinernematid Q1;


% MORTALITY
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D
10^0
10^1
10^2
10^3
10^4
DOSAGE (No NEMATODES)

TABLE X

Infectivity of *Heterorhabditis* spp., and *Steinernema* spp., for various insect larvae in sand[a]

Nematode	Strain	*Heliothis punctiger*[b]		*Calliphora vicina*[b]		*Calliphora stygia*[b]		*Lucilia cuprina*[b]		*Lucilia sericata*[b]		*Adoryphorus couloni*[c]		*Otiorhynchus sulcatus*[c]		*Sitona humeralis*[c]	
		LD_{50}	95% limits	LD_{50}	95% limits	LD_{50}	95% limits	LD_{50}	95% limits	LD_{50}	95% limits	LD_{50}	95% limits	LD_{50}	95% limits	LD_{50}	95% limits
Heterorhabditis sp.	D1	1	1-2	35	18-69	7	4-14	18	12-26	125	58-268	*		4	2-6	-	
Heterorhabditis sp.	V16	2	1-3	23	8-56			54	17-153	116	59-231	21	7-56	-		-	
H. bacteriophora		8	6-12	104	49-221			138	48-416	79	37-167	107	39-293	12	7-19	44	14-120
H. heliothidis	NC	2	1-2	774	355-1,840			295	101-1,030	316	179-557	79	38-163	-		-	
H. heliothidis	T327	2	1-2	357	167-791	519	223-1340	295	103-1,000	261	119-590	64	24-164	4	3-5	7	5-10
H. heliothidis	NZ	2	1-3	100	44-228			71	32-156	847	389-2,030	39	14-101	5	3-9	6	4-8
Steinernema sp.	Q393	2	1-4	835	373-2,110			499	187-1,630			1,200	265-17,900	-		-	
S. bibionis	N60	3	2-4	309	149-647	90	43-192	5,350	1,680-36,200	231	111-486	*		2,480	1,090-7,630	-	
S. bibionis	T335	9	5-16	1,560	610-5,580	74	39-143	106	48-234	163	83-321	*		1,570	996-2,510	60	33-160
S. feltiae	Agriotis	15	9-42	285	138-596	84	45-156	71	33-151	1,020	412-3,210	*		204	143-297	221	84-636
S. glaseri	KG	5	3-7	795	372-1,830			119	51-279	232	109-504	37	18-74	33	20-63	-	
		LD_{90}	95% limits	LD_{90}	95% limits	LD_{90}	95% limits	LD_{90}	95% limits	LD_{90}	95% limit	LD_{90}	95% limits	LD_{90}	95% limits	LD_{90}	95% limits
Heterorhabditis sp.	D1	8	5-20	291	133-1,150	53	25-217	369	215-744	1,900	754-8,940	*		30	18-72	-	
Heterorhabditis sp.	V16	12	7-25	1,020	322-7,670			5,240	1,260-72,000	1,030	460-4,120	1,430	407-13,500	-		-	
H. bacteriophora		49	27-144	1,480	599-6,720			13,700	3,000-234,000	1,100	450-4,980	7,030	1,830-78,200	113	59-344	3,560	922-41,000
H. heliothidis	NC	8	5-17	13,400	4,650-94,200			37,000	$6,660-1.1x10^6$	1,280	695-4,210	967	405-4,155	-		-	
H. heliothidis	T327	9	6-19	5,580	2,120-29,800	14,700	4,400-128,100	32,800	6,210-829,800	4,890	1,790-27,300	3,200	943-26,700	24	16-41	28	18-51
H. heliothidis	NZ	10	6-20	2,280	819-12,600			1,340	509-6,730	14,500	5,000-105,000	2,160	635-18,500	71	36-234	19	13-35
Steinernema sp.	Q393	64	22-1,820	17,700	5,640-149,900			35,300	7,480-687,900			$1.5x10^6$	$61,600-1.0x10^{10}$	-		-	
S. bibionis	N60	17	11-43	3,630	1,500-16,700	1,270	515-5,780	$2.8x10^6$	$2.4x10^5-3.9x10^8$	2,860	1,180-13,020	*		101,200	$23,100-2.8x10^6$	-	
S. bibionis	T335	100	41-751	67,300	$14,400-1.5x10^6$	518	245-1,970	1,980	747-10,100	1,350	610-5,370	*		22,600	11,300-68,000	933	283-15,700
S. feltiae	Agriotis	261	74-8,050	3,330	1,390-15,100	481	238-1,770	1,080	432-5,020	41,000	9,800-664,000	*		1,270	764-2,720	14,300	3,490-185,700
S. glaseri	KG	24	15-55	12,000	4,350-77,000			3,140	1,080-19,100	3,480	1,360-17,100	337	150-1,370	251	113-1,250	-	

[a] Individual insects in 80g sand, moisture content 7% (pF = 1.3)

[b] at 28°C

[c] at 18°C

* Mortality did not reach 50% at any dosage.

dosages of 10^4 *Heterorhabditis* infective juveniles. With the 10^4 dosage the characteristic red pigmentation normally produced by the bacterial symbiont was absent and cadavers were invariably fetid. In contrast, both *Heterorhabditis* spp. and *Steinernema* spp. reproduced effectively in *H. punctiger* cadavers following dosages of 2^0 to 2^5 and 2^1 and 2^5 infective juveniles respectively at 28°C.

Heterorhabditis spp. and *Steinernema* spp. were also able to reproduce in all insect cadavers at 18°C except *Heterorhabditis* sp. D1 which did not reproduce at any dosage.

DISCUSSION

Most previous studies on susceptibility of insects to *Heterorhabditis* and *Steinernema* nematodes have relied on exposure of a number of insects on moist filter paper to one or two species of nematode (Laumond *et al.* 1979; Poinar 1979). Although these studies have shown that a very large number of insect species is susceptible, they provided little or no information on the degree of susceptibility in natural conditions. The study reported here not only gives and indication of the degree of susceptibility of a number of different insects in more natural conditions but for the first time tests a variety of nematode species/strains.

Although all insect species tested were susceptible to each of the nematode species/strains, there were appreciable differences between the infectivities of the various nematode species/strains and between the susceptibilities of the various insect species.

The infectivity of most of the heterorhabditids was greater than that of the steinernematids for *O. sulcatus, H. punctiger, A. couloni,*

and *S. humeralis* at all dosages, and for *C. vicina, C. stygia, L. cuprina* and *L. sericata* at the lower dosages. Moreover, in the last four species of insects, only the heterorhabditids were able to reproduce effectively. The failure of *Steinernema* spp. to reproduce in the cadavers, like the failure of *Heterorhabditis* spp. when applied in heavy dosages, was due to the contamination of the cadaver by high populations of foreign micro-organisms (Molyneux *et al.* 1983). It is suggested here that when nematodes enter via the gut, as do *Steinernema* spp., or in very large numbers, they carry into the haemocoel so many contaminating micro-organisms that these are able to proliferate before symbiotic bacteria are able to inhibit their growth. The rapid growth of the foreign micro-organisms apparently leads to oxygen depletion and asphyxiation of the nematode as well as rendering the insect unsuitable as a growth medium nutritionally.

An important result of the present study is that even for a limited range of insect species, no one nematode was most infectious for all the insect hosts. Although *Heterorhabditis* sp. D1 was shown to be most infectious for *C. stygia* and *L. cuprina* and among the most infectious for *C. vicina, L. sericata, O. sulcatus, H. punctiger* and *G. mellonella*, it was by far the least infectious *Heterorhabditis* sp. strain for *A. couloni*.

Two of the nematode species/strains were originally obtained from pest insects tested in this study. These nematodes, *H. bacteriophora* and *S. bibionis* strain T335, were among the least infectious for the insects from which they were isolated (*H. punctiger* and *O. sulcatus*, respectively). Furthermore, *S. bibionis* strain N60, which was isolated from soil at a sheep campsite where *L. cuprina* larvae were likely to be

very common, was among the least infectious for this insect (Molyneux *et al.* 1983). In support of this, the N60 strain of *S. bibionis* was far more virulent against *L. sericata* than against *L. cuprina* (LD_{50} = 231 and 1,680 resp.; LD_{90} = 2,860 and 2.8 x 10^6 resp.). This finding has several important implications:

1) It invalidates any assumption that a nematode strain is likely to be the most infectious against its natural host; and
2) it indicates that in insect populations where nematodes are naturally present, better control might be achieved by the introduction of other species/strains.

Of more interest, however, is the possibility that continued association with a particular insect species leads to selection for reduction of infectivity for that insect species. This would parallel the reduction of virulence of other pathogens and parasites following prolonged association with a particular host species. Just as a well-evolved parasite does as little harm to its host as possible, it is likely that a well-evolved and adapted parasitoid (which by definition kills the host) does as little harm to the host population as possible (Bedding *et al.* 1983).

Unlike the other insects tested, *G. mellonella* larvae do not live in or pupate in soil. However, their susceptibility in this environment is interesting because of the importance of this insect in baiting soil samples for nematodes (Bedding and Akhurst 1975, Mráček 1982b). *G. mellonella* was the most susceptible insect for every species/strain of nematode. Indeed, the susceptibility of *G. mellonella* to all but one of the nematode species/strains was greater than that of any other insect species to any nematode species/strain (Fig. 30). *G. mellonella* larvae were so susceptible that even when there was only one infective

juvenile per container, parasitization was never less than 40%; with ten of the species/strains, one infective juvenile per container produced over 50% parasitization, while with *S. glaseri* and *H. heliothidis* strain NC, one infective juvenile per container produced over 90% parasitization.

From these tests, it can be seen (Figs. 30-33) that *S. feltiae* was the least infectious nematode for two of the insect species tested and was never the most infectious. Far more studies have been made to test the effectiveness of *S. feltiae* as an insect pathogen than of all other steinernematids and heterorhabditids put together (Poinar 1979, Gaugler 1981) and may have led to the rejection of the possibility of using nematodes to control particular pests. In the light of this study, together with the rapid progress that has been made in the last five years (Bedding 1981, Gaugler 1981), infective juvenile nematodes other than those of *S. feltiae* may well be used in the future for the control of soil-dwelling insect pests.

SYNTHESIS AND CONCLUSION

This study considers, for the first time, the behaviour and survival of the entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* within the soil environment in the presence and absence of a range of insect hosts. These genera were selected because, in contrast with other entomogenous nematodes, they are not host specific, can be mass produced economically *in vitro* and have the greatest potential for application in the biological control of various insect pests.

In preliminary studies, the cephalic region of adult nematodes was examined by scanning electron microscopy in an attempt to differentiate taxonomically the many species/strains. Although strain differences could not be detected the study did demonstrate various generic differences and the possibility of a new genus.

Examination of immature stages and the application of biochemical techniques e.g. electrophoresis might well resolve the difficulty in separating the various nematode strains. Correct identification of the different nematode species/strains is essential to the use of the appropriate nematode in biocontrol programmes.

Previous studies on the biology and ecology of heterorhabditids and steinernematids have invariably involved only one or two species of nematode. These investigations have been conducted under highly artificial laboratory conditions and, in particular, in the absence of the soil habitat. The soil factors investigated in the present study included a consideration of soil type, moisture and temperature. In common with many soil-dwelling nematodes, migration (measured in terms of infectivity) was restricted in soils with a high clay content, with the larger nematodes having the lowest level of infectivity. Although not measured directly, these results indicated the importance of soil

pore sizes in relation to size of nematode for nematode movement through the soil profile. Moisture potential (pF) was found to be a more meaningful factor than the often quoted moisture content and enabled nematode activity in different soil types to be compared. Moisture content *per se* does not take into account the effects of soil texture, pore size, particle size, organic matter, bulk density, surface area, etc. on the distribution of the soil water and therefore their influence on nematode movement. Nematodes from both genera displayed resistance to drying in a loamy sand and infected insect hosts at moisture potentials near the permanent wilting point of plants (pF 4.2). Nematode dissemination and infection of insect hosts is therefore possible in soils of similar texture in areas of low rainfall and no irrigation.

Nematode activity and survival was correlated with temperature and the optima for the different species/strains, though variable, reflected the geographic regions from which they were originally isolated. Of the two genera, the steinernematids were more active at lower temperatures. Although all nematodes tested were able to survive temporary periods of adversity, some species utilized special survival mechanisms. In the absence of insect hosts, some steinernematids became quiescent while the heterorhabditids were relatively short lived. For permanent establishment of these nematodes in the field, the heterorhabditids would be best suited to those areas where they would be in continual association with soil-dwelling insects susceptible to nematode infection.

Post-feeding third instar *L. cuprina* larvae were used in the study of those key soil factors that influenced nematode behaviour and survival because this stage of the life cycle pupariates in soil. The larvae

were particularly suited to experiments designed to investigate the effect of soil moisture on nematode infectivity because their inability to ingest soil particles and therefore nematodes, prevented passive entry of nematodes into the insect larvae.

Other factors such as soil particle size, organic matter content and water movement were not investigated but would be additional factors to consider for a better understanding of the nematode/soil habitat interaction.

Important differences between the two genera were observed in the nematode/insect bioassays. In general, the heterorhabditids were more effective: they infected many different hosts and different developmental stages within a particular host species; were more virulent and developed and reproduced inside cadavers more often than did the steinernematids. The ability of heterorhabditids to penetrate the outside cuticle and intersegmental membranes by abrasion allows penetration of insects that are either heavily sclerotised (e.g. pupae) have protective coverings over spiracles (e.g. scarab larvae) or whose anal and oral cavities are too small for nematode entry.

Nematode reproduction was affected adversely if population densities of foreign micro-organisms and/or nematodes were too high inside cadavers. In general, cuticular penetration allowed the heterorhabditids to reproduce more often because, unlike the steinernematids, the bacterial flora of the host gut was not always introduced into the insect's haemocoel. However, at high enough dosages both genera failed to develop and reproduce.

As a consequence of these observations, it will not be necessary to test all species/strains when selecting nematodes as possible biological

control agents for a particular pest insect. To minimize testing, those nematodes unsuited to the prospective host environment by virtue of temperature requirement could be eliminated and a preliminary scan at a dosage of 100 nematodes per insect may eliminate many other species/strains. The insect type and stage of development will determine which nematode group to use and the appropriate dosage rate to apply. The degree of insect susceptibility to nematode infection was described statistically and dosage rates determined using probit analysis. The correct dosage will ensure that not only is the immediate pest situation brought under control but that a residual nematode population will be established permanently in the field. This reservoir of biocontrol agents may help to check future insect pest populations.

Information on other factors responsible for the level of infectivity such as the attraction of nematodes to insects, the rate of nematode and host dispersal and host defense mechanisms should also be studied. These additional factors will help maximize the usage of these nematodes in field applications by understanding more fully the biology and ecology of these nematodes.

In the present study, nematode/insect bioassays were conducted in sand instead of soil. Sand was preferred to soil because it was less variable and provided a uniform and easily replicated method of testing. Furthermore, the behaviour and survival of the nematodes investigated was conducted under constant temperature, moisture and controlled experimental laboratory conditions. Although this type of approach is essential for our understanding of the interactions between the insect, nematode and soil system, it is only the first step. Nematode behaviour and survival under fluctuating temperatures and moisture levels and the

interaction between different nematode species and insect host species must also be considered. The effects of predators (e.g. collembola, monochid and dorylaimid nematodes, enchytraeids and fungi) and parasites (bacteria, protozoa and viruses) will also affect this nematode/insect interaction and should also be studied. A consideration and understanding of all these factors will enable realistic models to be developed for future diagnostic and advisory programmes relating to the usage of these nematodes in various insect pest situations.

Although heterorhabditids and steinernematids have not been used extensively in the field for the control of insect pests in soil, they have some distinct advantages over some of the more conventional control methods. In contrast with insecticides, they are insect specific, self-perpetuating, non-polluting, able to actively seek and locate the insect pest and maybe mass produced economically. Also, as yet, there is no record of any insect resistance to the nematode/bacterium complex.

In conclusion, this study has highlighted the need to consider and understand each of the interacting components, the insect, nematode and soil system in relation to key environmental factors and should contribute to the realization of using *Heterorhabditis* and *Steinernema* spp. as biocontrol agents of soil-dwelling insect pests.

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REFERENCES

AKHURST, R.J. (1980). Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. *J. Gen. Microbiol.* **121**: 303-309.

AKHURST, R.J. (1982a). A *Xenorhabdus* sp. (Eubacteriales : Enterobacteriaceae) symbiotically associated with *Steinernema kraussei* (Nematoda : Steinernematidae). *Revue de Nematologie* **5**: 277-280.

AKHURST, R.J. (1982b). Antibiotic activity of *Xenorhabdus* spp., bacteria symbiotically associated with insect pathogenic nematodes of the families Heterorhabditidae and Steinernematidae. *J. Gen. Microbiol.* **128**: 3061-3065.

AKHURST, R.J. (1983a). Taxonomic study of *Xenorhabdus*, a genus of bacteria symbiotically associated with insect pathogenic nematodes. *Int. J. Syst. Bacteriol.* **33**: 38-45.

AKHURST, R.J. (1983b). *Neoaplectana* species: specificity of association with bacteria of the genus *Xenorhabdus*. *Exp. Parasitol.* **55**: 258-263.

AKHURST, R.J. and BEDDING, R.A. (1978). A simple cross-breeding technique to facilitate species determination in the genus *Neoaplectana*. *Nematologica* **24**: 328-330.

ALL, J.N., SAUNDERS, M.C., DUTCHER, J.D. and JAVID, A.M. (1981). Susceptibility of grape root borer larvae, *Vitacea polistiformis* (Lepidoptera : Sesiidae) to *Neoaplectana carpocapsae* (Nematoda : Rhabditida) : potential of host kairomones for enhancement of nematode activity in grape vineyards. *Misc. Publ. Entomol. Soc. Am.* **12**: 9-14.

ALVEY, N.G. *et al.* (1977). Regression and generalised linear models. *In*: "GENSTAT: A General Statistical Program". Ch. 7, Rothamsted Experimental Station.

ANDERSON, G.L. (1978). Responses of dauer larvae of *Caenorhabditis elegans* (Nematoda : Rhabditidae) to thermal stress and oxygen deprivation. *Can. J. Zool.* **56**: 1786-1791.

ANDREADIS, T.G. and HALL, D.M. (1976). *Neoaplectana carpocapsae* : encapsulation in *Aedes aegypti* and changes in host hemocytes and hemolymph proteins. *Exp. Parasitol.* **39**: 252-261.

BEDDING, R.A. (1972). Biology of *Deladenus siricidicola* (Neotylenchidae) an entomophagous nematode parasitic in siricid woodwasps. *Nematologica* **18**: 482-493.

BEDDING, R.A. (1976). New methods increase the feasibility of using *Neoaplectana* spp. (Nematoda) for the control of insect pests. *Proc. 1st Int. Colloq. Invert. Pathol.* 250-254.

BEDDING, R.A. (1981). Low cost *in vitro* mass production of *Neoaplectana* and *Heterorhabditis* species (Nematoda) for field control of insect pests. *Nematologica* **27**: 109-114.

BEDDING, R.A. (1983). Large scale production, storage and transport of the insect parasitic nematodes *Neoaplectana* spp. and *Heterorhabditis* spp. *Ann. appl. Biol.* **104**.

BEDDING, R.A. (In press). Nematode parasites of Hymenoptera. *In*: "Plant and Insect Parasitic Nematodes". W.R. Nickle (Editor). Marcel Dekker, New York.

BEDDING, R.A. and AKHURST, R.J. (1975). A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* **21**: 109-110.

BEDDING, R.A. and MILLER, L.A. (1981a). Disinfesting blackcurrant cuttings of *Synanthedon tipuliformis*, using the insect parasitic nematode, *Neoaplectana bibionis*. *Environ. Entomol.* **10**: 449-453.

BEDDING, R.A. and MILLER, L.A. (1981b). Use of a nematode, *Heterorhabditis heliothidis*, to control black vine weevil, *Otiorhynchus sulcatus*, in potted plants. *Ann. appl. Biol.* **99**: 211-216.

BEDDING, R.A. and MOLYNEUX, A.S. (1982). Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda). *Nematologica* **28**: 354-359.

BEDDING, R.A., MOLYNEUX, A.S. and AKHURST, R.J. (1983). *Heterorhabditis* spp., *Neoaplectana* spp. and *Steinernema kraussei*: Interspecific and Intraspecific differences in infectivity for insects. *Exp. Parasitol.* **55**: 249-257.

BENHAM, G.S. Jr. and POINAR, G.O. Jr. (1973). Tabulation and evaluation of recent field experiments using the DD-136 strain of *Neoaplectana carpocapsae* Weiser : a review. *Exp. Parasitol.* **33**: 248-252.

BIRD, A.F. (1959). The attractions of roots to the plant parasitic nematodes *Meloidogyne javanica* and *M. hapla*. *Nematologica* **4**: 322-335.

BIRD, A.F. and AKHURST, R.J. (in press). The nature of the intestinal vescile in nematodes of the family Steinernematidae. *Int. J. Parasitol.*

BIRD, A.F. and WALLACE, H.R. (1965). The influence of temperature on *Meloidogyne hapla* and *M. javanica*. *Nematologica* **11**: 581-589.

BOVIEN, P. (1937). Some types of association between nematodes and insects. *Vidensk. Medd. Dansk Naturh. Foren Bd.* **101**: 1-114.

BURMAN, M. (1981). The pathogenicity of *Neoaplectana carpocapsae* Weiser (Nematoda) toward *Hylobius abietis* L. (Insecta). Ph.D. dissertation, University of Umea, Sweden.

BURMAN, M. (1982). *Neoaplectana carpocapsae* : toxin production by axenic insect parasitic nematodes. *Nematologica* **28**: 62-70.

BURMAN, M. and PYE, A.E. (1980a). *Neoaplectana carpocapsae* : respiration of infective juveniles. *Nematologica* **26**: 214-219.

BURMAN, M. and PYE, A.E. (1980b). *Neoaplectana carpocapsae* : movements of nematode populations on a thermal gradient. *Exp. Parasitol.* **49**: 258-265.

BURMAN, M., PYE, A.E. and NOJD, N.O. (1979). Preliminary field trial of the nematode *Neoaplectana carpocapsae* against larvae of the large pine weevil, *Hylobius abietis* (Coleoptera, Curculionidae). *Ann. Entomol. Fenn.* **45**: 88.

BURSELL, E. (1974). The insect and the external environment. *In*: "The physiology of insects". Vol II., M. Rockstein (Editor). Academic Press, New York and London.

BYERS, J.A. and POINAR, G.O., Jr. (1982). Location of insect hosts by the nematode, *Neoaplectana carpocapsae*, in response to temperature. *Behaviour* **79**: 1-10.

CHENG, H.H. and BUCHER, G.E. (1972). Field comparison of the neoaplectanid nematode DD-136 with diazinon for control of *Hylemya* spp. on tobacco. *J. Econ. Entomol.* **65**: 1761-1763.

CHILDS, E.C. (1940). The use of soil moisture characteristics in soil studies. *Soil Sci.* **50**: 239-252.

CHRISTIE, J.R. (1936). Life history of *Agamermis decaudata*, a nematode parasite of grasshoppers and other insects. *J. Agric. Res.* **52**: 161-198.

CLEARWATER, J.R. and WOUTS, W.M. (1980). Preliminary trials on the control of lemon tree borer with nematodes. In: *Proc. 33rd N.Z. Weed and Pest Control Conf.* pp. 133-135.

CROLL, N.A. (1967). Acclimatization in the eccritic thermal response of *Ditylenchus dipsaci*. *Nematologica* **13**: 385-389.

CROLL, N.A. (1970). *"The Behaviour of Nematodes : their activity, senses and response"*. 117pp. Edward Arnold, London.

CRUICKSHANK, R., DUGUID, J.P. and SWAIN, R.H.A. (eds.). (1970). *"Medical Micriobiology"*, 11th edn. Edinburgh, E. and S. Livingstone.

CURRIE, J.A. (1961). Gaseous diffusion in the aeration of aggregated soils. *Siol Sci.* **92**: 40-45.

DANILOV, L.G. (1978). Effect of biotic and abiotic factors on the migration activity of entomogenous nematodes (*Neoaplectana carpocapsae*, Weiser, 1955, *"Agriotos"* strain) in soil. *Biull. Vses. Nauch. Issl. Inst. Zash. Rast.* **43**: 21-27 (in Russian, English summary).

DeCONINCK, L. (1965). Classe des Nematodes : Generalites. *In*: "Fraite de Zoologie". P.P. Grasse (Editor). Vol. IV. 3-217. Masson et Cie, Paris.

DUSENBERY, D.B. (1980). Behaviour of free-living nematodes. *In*: "Nematodes as biological models". Zuckerman (Editor) Vol. 1, 127-158 pp.

DUSENBERY, D.B., ANDERSON, G.L. and ANDERSON, E.A. (1978). Thermal acclimation more extensive for behavioural parameters than for oxygen consumption in the nematode *Caenorhabditis elegans*. *J. Exp. Zool.* **206**: 191-198.

DUTKY, S.R. (1959). Insect microbiology. *Adv. Appl. Microbiol.* **1**: 175-200.

DUTKY, S.R. (1974). Nematode parasites, pp. 576-590. *In*: F.C. Maxwell and F.A. Harris (Editors), *Proc. Summer Inst. on Biol. Control of Plant Insects and Disease*. University Press Mississippi, Jackson. 647 pp.

DUTKY, S.R., THOMPSON, J.V. and CANTWELL, G.E. (1962). A technique for mass rearing the greater wax moth (Lepidoptera : Galleriidae). *Proc. Ent. Soc. Wash.* **64**: 56-58.

ELLENBY, C. (1969). Dormancy and Survival in Nematodes. *Symp. Soc. Exp. Biol.* **23**: 83-97.

FILIPJEV, I.N. (1934a). The classification of the free-living nematodes and their relation to parasitic nematodes. *Smithson. Misc. Coll.* **89**: 1-63.

FILIPJEV, I.N. (1934b). Miscellanea Nematologica. I. Eine neue Art der Gotting *Neoaplectana* Steiner nebst Bemerkunge uber die systematische Stellung der letzteren. *Magazin de Parasitologie de l'Institut Zoologique de l'Academie de l'USSR* **4**: 229-240.

FINNEY, D.J. (1971). "*Probit analysis*". 3rd Ed. Cambridge Uni. Press, London, England.

FINNEY, J.R. and MORDUE, W. (1976). The susceptibility of the elm bark beetle *Scolytus scolytus* to the DD-136 strain of *Neoaplectana* sp. *Ann. Appl. Biol.* **83**: 311-312.

FINNEY, J.R. and WALKER, C. (1977). The DD-136 strain of *Neoaplectana* sp. as a potential biological control agent for the European elm bark beetle, *Scolytus scolytus*. *J. Invert. Pathol.* **29**: 7-9.

FINNEY, J.R. and WALKER, C. (1979). Assessment of a field trial using the DD-136 strain of *Neoaplectana* sp. for the control of *Scolytus scolytus*. *J. Invert. Pathol.* **33**: 248-252.

FINNEY, J.R., LIM, K.P. and BENNETT, G.F. (1982). The susceptibility of the spruce budworm, *Choristoneura fumiferana* (Lepidoptera : Torticidae), to *Heterorhabditis heliothidis* (Nematoda : Heterorhabditidae) in the laboratory. *Can. J. Zool.* **60**: 958-961.

FRAENKEL, G. and BHASKARAN, C. (1973). Pupariation and pupation in cyclorrhaphous flies (Diptera): Terminology and Interpretation. *Ann. Ent. Soc. Am.* **66**: 418-422.

GAUGLER, R. (1981). Biological control potential of neoaplectanid nematodes. *J. Nematol.* **13**: 241-249.

GAUGLER, R. and BOUSH, G.M. (1978). Effects of ultraviolet radiation and sunlight on the entomogenous nematode, *Neoaplectana carpocapsae*. *J. Invert. Pathol.* **32**: 291-296.

GAUGLER, R. and BOUSH, G.M. (1979a). Laboratory tests on ultraviolet protectants of an entomogenous nematode. *Environ. Entomol.* **8**: 810-813.

GAUGLER, R. and BOUSH, G.M. (1979b). Nonsusceptibility of rats to the entomogenous nematode, *Neoaplectana carpocapsae*. *Environ. Entomol.* **8**: 658-660.

GAUGLER, R. and BOUSH, G.M. (1979c). Effects of gamma radiation on the entomogenous nematode, *Neoaplectana carpocapsae*. *J. Invert. Pathol.* **33**: 121-123.

GAUGLER, R., LeBECK, L., NAKAGAKI, B. and BOUSH, G.M. (1980). Orientation of the entomogenous nematode *Neoaplectana carpocapsae* to carbon dioxide. *Environ. Entomol.* **9**: 649-652.

GEORGIS, R. and HAGUE, N.G.M. (1981). A neoaplectanid nematode in the larch sawfly *Cephalcia lariciphila* (Hymenoptera : Pampiliidae). *Ann. Appl. Biol.* **99**: 171-177.

GEORGIS, R. and POINAR, G.O., Jr. (1983). Effect of soil texture on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematoda : Steinernematidae). *J. Nematol.* **15**: 308-311.

GIRTH, H.B., McCOY, E.E. and GLASER, R.W. (1940). Field experiments with a nematode parasite of the Japanese beetle. *N.J. Dept. Agric.* Circ. No. **317**: 21 pp.

GLASER, R.W. (1932). Studies on *Neoaplectana glaseri*, a nematode parasite of the Japanese beetle, *Popillia japonica*. *N.J. Dept. Agric.* Circ. No. **211**: 34 pp.

GLASER, R.W. and FARRELL, C.C. (1935). Field experiments with the Japanese beetle and its nematode parasite. *J. N.Y. Entomol. Soc.* **43**: 345-371.

GLASER, R.W., McCOY, E.E. and GIRTH, H.B. (1940). The biology and economic importance of a nematode parasite in insects. *J. Parasitol.* **26**: 479-495.

GLASER, R.W., McCOY, E.E. and GIRTH, H.B. (1942). The biology and culture of *Neoaplectana chresima*, a new nematode parasitic in insects. *J. Parasitol.* **28**: 123-126.

GÖTZ, P., BOMAN, A. and BOMAN, H.G. (1981). Interactions between insect immunity and an insect-pathogenic nematode with symbiotic bacteria. *Proc. R. Soc. Lond., Series B* **212**: 333-350.

GRANDISON, G.S. (1973). Soil moisture and nematode parasitism of plants. *Proc. soil and plant water symp. N.Z.* **96**: 115-119.

HACKETT, K.J. and POINAR, G.O. Jr. (1973). The ability of *Neoaplectana carpocapsae* Weiser (Steinernematidae : Rhabditoidea) to infect adult honeybees *Apis mellifera* (Apidae : Hymenoptera). *Am. Bee J.* **113**: 100.

HARA, A.H., LINDEGREN, J.E. and KAYA, H.K. (1981). Monoxenic mass production of the entomogenous nematode, *Neoaplectana carpocapsae* Weiser, on dog food/agar medium. *USDA/SEA, AAT-W-16*. 8 pp.

HARLAN, D.P., DUTKY, S.R., PADGETT, G.R., MITCHELL, J.A., SHAW, Z.A. and BARTLETT, F.J. (1971). Parasitism of *Neoaplectana dutkyi* in white-fringed beetle larvae. *J. Nematol.* **3**: 280-283.

HEDGECOCK, E.M. and RUSSELL, R.L. (1975). Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. *Proc. Nat. Acad. Sci. U.S.A.* **72**: 4061-4065.

HOUSE, H.L., WELCH, H.E. and CLEUGH, T.R. (1965). Food medium of prepared dog biscuit for the mass production of the nematode DD-136 (Nematoda : Steinernematidae). *Nature* **206**: 847.

HOY, J.M. (1954). The biology and host range of *Neoaplectana leucaniae* a new species of insect-parasitic nematode. *Parasitology* **44**: 392-399.

JACKSON, G.J. (1962). The parasitic nematode, *Neoaplectana glaseri* in axenic culture. II. Initial results with defined media. *Exp. Parasitol.* **12**: 25-32.

JACKSON, G.J. (1973). The aging of *Neoaplectana glaseri*. *Proc. Helminth. Soc. Wash.* **40**: 74-76.

JACKSON, G.J. and MOORE, G.E. (1969). Infectivity of nematodes, *Neoaplectana* species for the larvae of the weevil *Hylobius pales*. after rearing in species isolation. *J. Invert. Pathol.* **14**: 194-198.

JACKSON, T.A., BEDDING, R.A., TROUGHT, T.E.T., KAIN, W.M. and EAST, R. (1981). The potential use of nematodes for the control of pasture pests. *Proc. 34th N.Z. Weed and Pest Control Conf.* 170-172.

JAQUES, R.P. (1967). Mortality of five apple insects induced by the nematode DD-136. *J. Econ. Entomol.* **60**: 741-743.

JAQUES, R.P., STULTZ, H.T. and HUSTON, F. (1968). The mortality of the pale apple leafroller and winter moth by fungi and nematodes applied to soil. *Can. Entomol.* **100**: 813-818.

KABLE, P.F. and MAI, W.F. (1968). The influence of soil moisture on *Pratylenchus penetrans*. *Nematologica* **14**: 101-122.

KAIN, W.M., BEDDING, R.A. and Van Der MESPEL, C.J. (1982). Preliminary evaluations of parasitic nematodes for grass grub (*Costelytra zealandica* [White]) control in central Hawke's Bay of New Zealand. *N.Z. J. of Exp. Agric.* **10**: 447-450.

KAIN, W.M., WYETH, T.K. and KALE, A.J. (1981). Preliminary field studies on the use of an indigenous parasitic nematode for control of porina. *Proc. 34th N.Z. Weed and Pest Control Conf.*: 173-175.

KAMIONEK, M. (1977). Pathogenicity of the nematode *Neoaplectana carpocapsae* Weiser in relation to different hosts. *Bull. Acad. Pol. Sci.* **25**: 243-246.

KAMIONEK, M. and SANDNER, H. (1974). The survival of the larvae of *Neoaplectana carpocapsae* outside an aquatic environment under various conditions of temperature and humidity. *Wiad. Parazytol.* **20**: 723.

KAMIONEK, M., MASLANA, I. and SANDNER, H. (1974). The survival of invasive larvae of *Neoaplectana carpocapsae* Weiser in a waterless environment under various conditions of temperature and humidity. *Zesz. Probl. Post. Nauk Roln.* **154**: 409-412.

KAYA, H.K. (1977). Development of the DD-136 strain of *Neoaplectana carpocapsae* at constant temperatures. *J. Nematol.* **9**: 346-349.

KAYA, H.K. (1978). Infectivity of *Neoaplectana carpocapsae* and *Heterorhabditis heliothidis* to pupae of the parasite *Apanteles militaris*. *J. Nematol.* **10**: 241-244.

KAYA, H.K. and GRIEVE, B.J. (1982). The nematode *Neoaplectana carpocapsae* and the beet armyworm *Spodoptera exigua* : infectivity of prepupae and pupae in soil and of adults during emergence from soil. *J. Invert. Pathol.* **39**: 192-197.

KAYA, H.K. and HARA, A.H. (1981). Susceptibility of various species of lepidopterous pupae to the entomogenous nematode *Neoaplectana carpocapsae*. *J. Nematol.* **13**: 291-294.

KHAN, A., BROOKS, W.M. and HIRSCHMANN, H. (1976). *Chromonema heliothidis* n. gen., n. sp. (Steinernematidae : Nematoda), a parsite of *Heliothis zea* (Noctuidae : Lepidoptera), and other insects. *J. Nematol.* **8**: 159-168.

KUIPER, K. and DE LEEUW, W.P. (1963). Landbouwpoederkalk als nematicide. *Meded. Landbouwhogesch. Gent.* **28**: 618-622.

LAM, A.B.Q. and WEBSTER, J.M. (1972). Effect of the DD-136 nematode and of a B-exotoxin preparation of *Bacillus thuringiensis* var. *thuringiensis* on leatherjackets, *Tipula paludosa* larvae. *J. Invert. Pathol.* **20**: 141-149.

LAUMOND, C., MAULÉON, H. and KERMARREC, A. (1979). New data on the host range and parasitism of the entomophilic nematode *Neoaplectana carpocapsae*. *Entomophaga* **24**: 13-27 (in French, English summary).

LEWIS, L.C. and RAUN, E.S. (1978). Laboratory and field evaluation of the DD-136 strain of *Neoaplectana carpocapsae* for control of the European corn borer, *Ostrinia nubilalis*. *Iowa State J. Res.* **52**: 391-396.

LINDEGREN, J.E., CURTIS, C.E. and POINAR, G.O. Jr. (1978). Parasitic nematode seeks out navel orangeworm in almond orchards. *Calif. Agric.* **32**: 10-11.

LINDEGREN, J.E., YAMASHITA, T.T. and BARNETT, W.W. (1981). Parasitic nematode may control carpenterworm in fig trees. *Calif. Agric.* **35**: 25-26.

LYSENKO, O. (1981). Principles of pathogenesis of insect bacterial diseases as exemplified by the non-spore forming bacteria. *In*: E.W. Davidson (Editor). *Pathogenesis of Invertebrate Microbiol Diseases*. Allanheld, Osmum & Co., Totowa, N.J.

MACKERRAS, M.J. (1933). Observations on the life-histories, nutritional requirements and fecundity of blowflies. *Bull. Ent. Res.* **24**: 353-362.

MacVEAN, C.M., BREWER, J.M. and CAPINERA, J.L. (1982). Field tests of antidesiccants to extend the infection period of an entomogenous nematode, *Neoaplectana carpocapsae*, against the Colorado potato beetle. *J. Econ. Entomol.* **75**: 97-101.

McCOY, E.E. and GIRTH, H.B. (1938). The culture of *Neoaplectana glaseri* on veal pulp. *N.J. Dept. Agric., Bureau of Plant Industry*. Circ. No. **285**: 1-12.

McILVAINE, T.C. (1921). A buffer solution for colorimetric comparison. *J. Biol. Chem.* **49**: 183-187.

McINTYRE, D.S. (1974). Water retention and the moisture characteristic. *In*: "Methods for Analysis of Irrigated Soils". J. Loveday (Editor). *Tech. Comm.* No 54: C.A.B. p. 108.

MILLER, L.A. and BEDDING, R.A. (1982). Field testing of the insect parasitic nematode, *Neoaplectana bibionis* (Nematoda : Steinernematidae) against currant borer moth, *Synanthedon tipuliformis* (Lep. : Sesiidae) in blackcurrants. *Entomophaga* **27**: 109-114.

MILSTEAD, J.E. (1981). Influence of temperature and dosage on mortality of seventh instar larvae of *Galleria mellonella* (Insecta : Lepidoptera) caused by *Heterorhabditis bacteriophora* (Nematoda : Rhabditoidea) and its bacterial associate *Xenorhabdus luminescens*. *Nematologica* **27**: 167-171.

MILSTEAD, J.E. and POINAR, G.O. (1978). A new entomogenous nematode for pest management systems. *Calif. Agri.* **32**: 12.

MOLYNEUX, A.S., BEDDING, R.A. and AKHURST, R.J. (1983). Susceptibility of larvae of the sheep blowfly *Lucilia cuprina* to various *Heterorhabditis* spp., *Neoaplectana* spp., and an undescribed steinernematid (Nematoda). *J. Invert. Pathol.* **42**: 1-7.

MOORE, G.E. (1965). The bionomics of an insect-parasitic nematode. *J. Kans. Entomol. Soc.* **38**: 101-105.

MOORE, G.E. (1970). *Dendroctonus frontalis* infection by the DD-136 strain of *Neoaplectana carpocapsae* and its bacterium complex. *J. Nematol.* **2**: 341-344.

MOORE, G.E. (1973). Moisture requirements of the DD-136 strain of *Neoaplectana carpocapsae* (Nematoda : Rhabditida) as related to host infection. *Exp. Parasitol.* **33**: 207-211.

MORGAN, G.T. and MacLEAN, A.A. (1968). Influence of soil pH on an introduced population of *Pratylenchus penetrans*. *Nematologica*. **14**: 311-315.

MOYLE, P.L. and KAYA, H.K. (1981a). Dispersal and infectivity of the entomogenous nematode, *Neoaplectana carpocapsae* Weiser (Rhabditida : Steinernematidae) in sand. *J. Nematol*. **13**: 419-421.

MOYLE, P.L. and KAYA, H.K. (1981b). Susceptibility of pupae of two cocoon-forming lepidopterous species to the entomogenous nematode *Neoaplectana carpocapsae* (Rhabditida : Steinernematidae). *J. Nematol*. **13**: 419-421.

MRÁČEK, Z. (1977). *Steinernema kraussei*, a parasite of the body cavity of the sawfly, *Cephalcia abietis*, in Czechoslovakia. *J. Insect Pathol*. **30**: 87-94.

MRÁČEK, Z. (1980). The use of "*Galleria* traps" for obtaining nematode parasites of insects in Czechoslovakia (Lepidoptera : Nematoda, Steinernematidae). *Acta Entomol. Bohem*. **77**: 378-382.

MRÁČEK, Z. (1982a). Horizontal distribution in soil, and seasonal dynamics of the nematode *Steinernema kraussei*, a parasite of *Cephalcia abietis*. *Z. Ang. Entomol*. **94**: 110-112.

MRÁČEK, Z. (1982b). Estimate of the number of infective larvae of *Neoaplectana carpocapsae* (Nematoda : Steinernematidae) in a soil sample. *Nematologica* **28**: 303-306.

MRÁČEK, Z. and WEISER, J. (1979). The head papillae of the Steinernematidae. *J. Invert. Pathol*. **34**: 310-311.

MRÁČEK, Z., GERDIN, S. and WEISER, J. (1981). Head and cuticular structures of some species in the family Steinernematidae (Nematoda). *Nematologica* **27**: 443-448.

NORRIS, K.R. and UPTON, M.S. (1974). *"The collection and preservation of insects"*. The Aust. Ent. Soc. Inc. Watson Ferguson and Co., Brisbane, Australia.

OBENDORF, D.L., PEEL, B., AKHURST, R.J. and MILLER, L.A. (1983). Non-susceptibility of mammals to the entomopathogenic bacterium *Xenorhabdus nematophilus*. *Environ. Entomol.* **12**: 368-370.

OBIAMIWE, B.A. and MacDONALD, W.W. (1973). A new parasite of mosquitoes *Reesimermis muspratti* sp. nov. (Nematoda : Mermithidae), with notes on its life cycle. *Ann. Trop. Med. and Parasitol.* **67**: 439-444.

ORCHISTON, H.D. (1953). Adsorption of water vapor. I. Soils at 25°C. *Soil Sci.* **76**: 453-465.

PETERS, B.G. (1953). Vertical migration of potato root eelworm. *J. Helminth.* **27**: 107-112.

PETERSEN, J.J. and CHAPMAN, H.C. (1970). Parasitism of *Anopheles* mosquitoes by a *Gastromermis* sp. (Nematoda : Mermithidae) in southwestern Louisiana. *Mosquito News* **30**: 420-424.

PIPER, C.S. (1947). *"Soil and Plant Analysis"*. Publishers Inc., New York, U.S.A.

POINAR, G.O., Jr. (1966). The presence of *Achromobacter nematophilus* in the infective stage of a *Neoaplectana* sp. (Steinernematidae : Nematoda). *Nematologica* **12**: 105-108.

POINAR, G.O., Jr. (1967). Description and taxonomic position of the DD-136 nematode (Steinernematidae : Rhabditoidea) and its relationship to *Neoaplectana carpocapsae* Weiser. *Proc. Helminthol. Soc. Wash.* **34**: 199-209.

POINAR, G.O., Jr. (1968). *Hydromermis conopophaga* n. sp., parasitizing midges (Chironomidae) in Calfornia. *Ann. entom. Soc. Am.* **61**: 593-598.

POINAR, G.O., Jr. (1971). Use of nematodes for microbial control of insects. *In*: "Microbial Control of Insects and Mites". H.D. Burgess and N.W. Hussey (Editors). Academic Press, London. 181-203 pp.

POINAR, G.O., Jr. (1972). Nematodes as facultative parasites of insects. *Ann. Rev. Ent.* **17**: 103-122.

POINAR, G.O., Jr. (1975). Description and biology of a new insect parasitic rhabditoid, *Heterorhabditis bacteriophora* n. gen., n. sp. (Rhabditida : Heterorhabditidae n. fam.). *Nematologica* **21**: 463-470.

POINAR, G.O., Jr. (1979). *"Nematodes for Biological Control of Insects"*. CRC Press, Boca Raton, Fl., U.S.A.

POINAR, G.O., Jr. and DONCASTER, C.C. (1965). The penetration of *Tripius sciarae* (Bovien) (Sphaerulariidae : Aphelenchoidea) into its insect host, *Bradysia paupera* Tuom. (Mycetophilidae : Diptera). *Nematologica* **11**: 73-78.

POINAR, G.O., Jr. and HIMSWORTH, P.T. (1967). *Neoaplectana* parasitism of larvae of the greater wax moth, *Galleria mellonella*. *J. Invert. Pathol.* **9**: 241-246.

POINAR, G.O., Jr., and LEUTENEGGER, R. (1968). Anatomy of the infective and normal third-stage juveniles of *Neoaplectana carpocapsae* Weiser (Steinernematidae : Nematoda). *J. Parasitol.* **54**: 340-350.

POINAR, G.O., Jr. and THOMAS, G.M. (1965). A new bacterium *Achromobacter nematophilus* sp. nov. (Achromobacteriaceae : Eubacteriales) associated with a nematode. *Int. Bull. Bacteriol. Nomen. Taxon.* **15**: 249-252.

POINAR, G.O., Jr. and THOMAS, G.M. (1966). Significance of *Achromobacter nematophilus* Poinar and Thomas (Achromobacteracae : Eubacteriales) in the development of the nematode, DD-136 (*Neoaplectana* sp. Steinernematidae). *Parasitology* **56**: 385-390.

POINAR, G.O., Jr. and THOMAS, G.M. (1967). The nature of *Achromobacter nematophilus* as an insect pathogen. *J. Invert. Pathol.* **9**: 510-514.

POINAR, G.O., Jr. HESS, R. and THOMAS, G.M. (1980). Isolation of defective bacteriophages from *Xenorhabdus* spp. (Enterobacteriaceae). *IRCS Med. Sci.* **8**: 141.

POINAR, G.O., Jr. THOMAS, G.M. and HESS, R. (1977). Characteristics of the specific bacterium associated with *Heterorhabditis bacteriophora* (Heterorhabditidae : Rhabditida). *Nematologica* **23**: 97-102.

POINAR, G.O., Jr., THOMAS, G.M., PRESSER, S.B. and HARDY, J.L. (1982). Inoculation of entomogenous nematodes, *Neoaplectana* and *Heterorhabditis* and their associated bacteria, *Xenorhabdus* spp. into chicks and mice. *Environ. Entomol.* **11**: 137-138.

PYE, A.E. and BURMAN, M. (1978). *Neoaplectana carpocapsae* : infection and reproduction in large pine weevil larvae, *Hylobius abietis*. *Exp. Parasitol.* **46**: 1-11.

PYE, A.E. and BURMAN, M. (1981). *Neoaplectana carpocapsae*: nematode accumulations on chemical and bacterial gradients. *Exp. Parasitol.* **51**: 13-20.

REED, E.M. and CARNE, P.B. (1967). The suitability of a nematode (DD-136) for the control of some pasture insects. *J. Invert. Pathol.* **9**: 196-204.

REED, E.M. and WALLACE, H.R. (1965). Leaping locomotion by an insect parasitic nematode. *Nature* (London) **206**: 210-211.

RUSSELL, E.W. (1973). "*Soil Conditions and Plant Growth*". Longman Ltd., London.

SANDNER, H., and STANUSZEK, S. (1971). Comparative research on the effectiveness and production of *Neoaplectana carpocapsae*. *s.l. Zesz. Probl. Poste. Nauk Roln.* **121**: 209-226.

SAUNDERS, M.C. and ALL, J.N. (1982). Laboratory extraction methods and field detection of entomophilic rhabditoid nematodes from soil. *Environ. Entomol.* **11**: 1164-1165.

SCHMIDT, J. and ALL, J.N. (1978). Chemical attraction of *Neoaplectana carpocapsae* (Nematoda : Steinernematidae) to insect larvae. *Environ. Entomol.* **7**: 605-607.

SCHMIDT, J. and ALL, J.N. (1979). Attraction of *Neoaplectana carpocapsae* (Nematoda : Steinernematidae) to common excretory products of insects. *Environ. Entomol.* **8**: 55-61.

SCHMIEGE, D.C. (1963). The feasibility of using a neoaplectanid nematode for control of some forest insect pests. *J. Econ. Entomol.* **56**: 427-431.

SEINHORST, J.W. (1950). De betekenis van de toestand van de grond voor het optreden van aantasting door het stengelaaltje (*Ditylenchus dipsaci* [Kuhn] Filipjev). *Tydschr. Plziekt.* **56**: 289-348. (English summary pp. 345-347).

SEXTON, S.B. and WILLIAMS, P. (1981). A natural occurrence of parasitism of *Graphognathus leucoloma* (Boheman) by the nematode *Heterorhabditis* sp. *J. Aust. ent. Soc.* **20**: 253-255.

SHOREY, H.H. and HALE, R.L. (1965). Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. *J. Econ. Ent.* **58**: 522-524.

SILVERMAN, J., PLATZER, E.G. and RUST, M.K. (1982). Infection of the rat flea *Ctenoceophalides felis* (Bouche) by *Neoaplectana carpocapsae* Weiser. *J. Nematol.* **14**: 394-397.

SIMONS, W.R. (1973). Nematode survival in relation to soil moisture. *Meded. Landbouwhegesch. Wageningen* **73**: 1-85.

SIMONS, W.R. (1981). Biological control of *Otiorhynchus sulcatus* with Heterorhabditid nematodes in the glasshouse. *Neth. J. Pl. Path.* **87**: 149-158.

SIMONS, W.R. and POINAR, G.O., Jr. (1973). The ability of *Neoaplectana carpocapsae* (Steinernematidae : Nematoda) to survive extended periods of desiccation. *J. Invert. Pathol.* **22**: 228-230.

STACE, H.C.T., HUBBLE, G.D., BREWER, R., NORTHCOTE, K.H., SLEEMAN, J.R., MULCAHY, M.J. and HALLSWORTH, E.G. (1968). "*A Handbook of Australian Soils*". (Rellin Tech. Publs., Glenside, S. Aust.).

STANUSZEK, S. (1972). Revision of the genus *Neoaplectana* Steiner 1929 (Rhabditoidea : Steinernematidae). *Abst. 11th Int. Symp. Nemat., Reading*, 69-70.

STANUSZEK, S. (1974). *Neoaplectana feltiae* complex (Nematoda : Rhabditoidea, Steinernematidae) its taxonomic position within the genus *Neoaplectana* and intraspecific structure. *Zesz. probl. Poste. Nauk Roln.* **154**: 331-359.

STEINER, G. (1923). *Aplectana kraussei* n. sp. einer in der Blattwespe Lyda sp. parasitierende Nematodenform, nebst Bemerkungen uber das Seitenorgan der parasitischen Nematoden. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt.* **59**: 14-18.

STEINER, G. (1929). *Neoaplectana glaseri* n. g., n. sp. (Oxyuridae) a new nemic parasite of the Japanese beetle (*Popillia japonica* Newm.). *J. Wash. Acad. Sci.* **19**: 436-440.

SWAIN, R.B. (1943). Nematode parasites of the white-fringed beetles. *J. Econ. Entomol.* **36**: 671-673.

THOMAS, G.M. and POINAR, G.O. (1979). *Xenorhabdus* gen. nov., a genus of entompathogenic, nematophilic bacteria of the family Enterobacteriaceae. *Int. J. Syst. Bacteriol.* **29**: 352-360.

THOMASON, J., VAN GUNDY, S.D. and KIRKPATRICK, J.D. (1964). Motility and infectivity of *Meloidogyne javanica* as affected by storage time and temperature in water. *Phytopathology* **54**: 192-195.

TRAVASSOS, L. (1927). Sobre o Genera *Oxystomatium*, Boletim Biologico. *Trabalho do Laboratorio de Parasitologia da Faculdade de Medicina de Sao Paulo, Brasil* **5**: 20-21.

TRIGGIANI, O. and POINAR, G.O., Jr. (1976). Infection of adult lepidoptera by *Neoaplectana carpocapsae* (Nematoda). *J. Invert. Pathol.* **27**: 413-414.

TUCKER, B.M. and BEATTY, H.J. (1974). pH, conductivity and chlorides. *In*: "Methods for analysis of irrigated soils". J. Loveday (Editor). *Tech. Comm.* No. **54**: C.A.B. p. 108.

TURCO, C.P., THAMES, W.H., Jr. and HOPKINS, S.H. (1971). On the taxonomic status and comparative morphology of the species of the genus *Neoaplectana* Steiner (Neoaplectanidae : Nematoda). *Proc. Helminth. Soc. Wash.* **38**: 68-79.

VAN BRACHT, W. (cited in Poinar, G.O. [1979]). "*Nematodes for Biological Control of Insects*". CRC Press, Boca Raton, Fl., U.S.A. p. 169.

VAN GUNDY, S.D. (1965). Nematode behaviour. *Nematologica* **11**: 19-32.

VAN GUNDY, S.D., BIRD, A.F. and WALLACE, H.R. (1967). Aging and starvation in larvae of *Meloidogyne javanica* and *Tylenchulus semipenetrans*. *Phtyopathology* **57**: 559-571.

VEREMCHUK, G.V. (1977). *Neoaplectana* spp. and the biological control of insects. *Zascjota Rastenit* **8**: 31-52 (In Russian).

WALLACE, H.R. (1958a). Movement of eelworms. I. The influence of pore size and moisture content of the soil on the migration of larvae of the beet eelworm, *Heterodera schachtii* Schmidt. *Ann. appl. Biol.* **46**: 74-85.

WALLACE, H.R. (1958b). Movement of eelworms. II. A comparative study of the movement in soil of *Heterodera schachtii* Schmidt and of *Ditylenchus dipsaci* (Kuhn) Filipjev. *Ann. appl. Biol.* **46**: 86-94.

WALLACE, H.R. (1958c). Movement of eelworms. III. The relationship between eelworm length, activity and mobility. *Ann. appl. Biol.* **46**: 662-668.

WALLACE, H.R. (1959a). Movement of eelworms. IV. The influence of water percolation. *Ann. appl. Biol.* **47**: 131-139.

WALLACE, H.R. (1959b). Movement of eelworms. V. Observations on *Aphelenchoides ritzema-bosi* (Schwartz, 1912) Steiner, 1932 on florist's chrysanthemums. *Ann. appl. Biol.* **47**: 350-360.

WALLACE, H.R. (1960). Movement of eelworms. VI. The influence of soil type, moisture gradients and host plant roots on the migration of the potato root eelworm *Heterodera rostochiensis* Wollenweber. *Ann. appl. Biol.* **48**: 107-120.

WALLACE, H.R. (1961). The bionomics of the free-living stages of zooparasitic and phytoparasitic nematodes - a critical survey. *Helminth. Abstr. (a review)* **30**: 1-22.

WALLACE, H.R. (1962). Observations on the behaviour of *Ditylenchus dipsaci* in soil. *Nematologica* **7**: 91-101.

WALLACE, H.R. (1963). *"The Biology of Plant Parasitic Nematodes"*. Edward Arnold, London.

WALLACE, H.R. (1966). Factors influencing the infectivity of plant parasitic nematodes. *Proc. Roy. Soc., B* **164**: 592-614.

WALKLEY, A. and BLACK, I.A. (1934). cited in PIPER, C.S. (1947). *"Soil and Plant Analysis"*. Interscience Publishers Inc., New York, U.S.A.

WEBSTER, J.M. (1964). The effect of storage conditions on the infectivity of narcissus stem eelworms. *Nature (London)* **202**: 571-575.

WEBSTER, J.M. (1972). Nematodes and Biological Control. *In*: "Economic Nematology". J.M. Webster (Editor). *Academic Press, London*. 469-496 pp.

WEBSTER, J.M. (1973). Manipulation of environment to facilitate use of nematodes in biocontrol of insects. *Exp. Parasitol.* **33**: 197-206.

WEBSTER, J.M. (1980). Biocontrol : The potential of entomophilic nematodes in insect management. *J. Nematol.* **12**: 270-278.

WEBSTER, J.M. and BRONSKILL, J.F. (1968). Use of Gelgard 'M' and an evaporation retardant to facilitate control of larch sawfly by a nematode-bacterium complex. *J. Econ. Entomol.* **61**: 1370-1373.

WEISER, J. (1966). Nemoci hmyzu. Academia, Prague. 554 pp.

WELCH, H.E. (1965). Entomophilic nematodes. *Ann. rev. Ent.* **10**: 275-302.

WELCH, H.E. and BRIAND, L.J. (1961). Tests of the nematodes DD-136 and an associated bacterium for control of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). *Can. Entomol.* **93**: 759-763.

WELCH, H.E. and BRONSKILL, J.F. (1962). Parasitism of mosquito larvae by the nematode DD-136 (Nematoda : Neoaplectanidae). *Can. J. Zool.* **40**: 1263-1268.

WHITEHEAD, A.G. and HEMMING, J.R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Ann. Appl. Biol.* **55**: 25-38.

WIGGLESWORTH, V.B. (1972). *"The Principles of Insect Physiology"*. Halsted Press, John Wiley & Sons, Inc. New York.

WILLIAMS, J., PREBBLE, R.E., WILLIAMS, W.T. and HIGNETT, C.T. (1983). The influence of texture, structure and clay mineralogy on the soil moisture characteristic. *Aust. J. Soil Res.* **21**: 15-32.

WOUTS, W.M. (1979). The biology and life cycle of a New Zealand population of *Heterorhabditis heliothidis* (Heterorhabditidae). *Nematologica* **25**: 191-202.

WOUTS, W.M. (1980). Biology, life cycle and redescription of *Neoaplectana bibionis* Bovien, 1937 (Nematoda : Steinernematidae). *J. Nematol.* **12**: 62-72.

WOUTS, W.M., MRÁČEK, Z., GERDIN, S. and BEDDING, R.A. (1982). *Neoaplectana* Steiner, 1929 a junior synonym of *Steinernema* Travassos, 1927 (Nematoda : Rhabditida). *Syst. Parasitol.* **4**: 147-154.

WRIGHT, K.A. (1980). Nematode sense organs. *In*: "Nematodes as biological models". (B.M. Zuckerman, Editor). Vol 2, pp. 237-295.

APPENDIX A

PUBLICATIONS ARISING FROM STUDIES DESCRIBED IN THIS THESIS

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MOLYNEUX, A.S., BEDDING, R.A. and AKHURST, R.J. (1983). Susceptibility of larvae of the sheep blowfly *Lucilia cuprina* to various *Heterorhabditis* spp., *Neoaplectana* spp., and an undescribed steinernematid (Nematoda). *J. Invert. Pathol.* **42**: 1-7.

BEDDING, R.A. and MOLYNEUX, A.S. (1982). Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae : Nematoda). *Nematologica* **28**: 354-359.

BEDDING, R.A., MOLYNEUX, A.S. and AKHURST, R.J. (1983). *Heterorhabditis* spp., *Neoaplectana* spp. and *Steinernema kraussei* : Interspecific and intraspecific differences in infectivity for insects. *Exp. Parasitol.* **55**: 249-257.

APPENDIX B

FULL DETAILS OF DATA USED TO COMPARE DIFFERENCES IN INFECTIVITY BETWEEN VARIOUS *HETERORHABDITIS* AND *STEINERNEMA* SPECIES/STRAINS FOR POST-FEEDING THIRD INSTAR *LUCILIA CUPRINA* STRAIN AM-3 LARVAE IN SAND AT VARIOUS TEMPERATURES

TABLE I

Tests of coincidence between pairs of probit lines for differences in infectivity between various *Heterorhabditis* and *Steinernema* species/strains for post-feeding third instar *Lucilia cuprina* strain AM-3 larvae in sand[a] at various temperatures.

Data sets	Temperature °C	Common line Degrees of Freedom	Common line Deviance	Individual line Degrees of Freedom	Individual line Deviance
Steinernema bibionis T335 vs. *Steinernema bibionis* N60	18	14	82.65	12	27.07
	23	14	101.60	12	15.46
	28	14	441.40	12	32.96
Heterorhabditis heliothidis T327 vs. *Heterorhabditis heliothidis* NC	18	14	43.56	12	15.44
	23	14	61.72	12	25.85
	28	14	19.04	12	18.33
Heterorhabditis heliothidis T327 vs. *Heterorhabditis heliothidis* NZ	18	14	169.70	12	18.18
	23	14	71.74	12	22.20
	28	14	24.86	12	20.55
Heterorhabditis heliothidis T327 vs. *Steinernema bibionis* T335	18	14	32.46	12	26.23
	23	14	34.48	12	25.82
	28	14	25.11	12	12.34
Heterorhabditis heliothidis T327 vs. *Steinernema glaseri* KG	18	14	48.74	12	19.01
	23	14	28.39	12	20.69
	28	14	34.24	12	15.35
Heterorhabditis sp. D1 vs. *Steinernema glaseri* KG	18	14	123.80	12	28.74
	23	14	20.69	12	12.54
	28	14	53.42	12	28.84
Heterorhabditis sp. D1 vs. *Steinernema bibionis* T335	18	14	223.60	12	35.97
	23	14	27.92	12	17.67
	28	14	140.20	12	25.83

[a] Individual *L. cuprina* larvae in 80 g fine sand, moisture content 7% (pF = 1.3).

APPENDIX C

FULL DETAILS OF DATA REGARDING THE NUMBER OF *HETERORHABDITIS* AND *STEINERNEMA* INFECTIVE JUVENILE NEMATODES RECOVERED FROM SAND (pF=1.3) AFTER STORAGE FOR 2^0 TO 2^5 WEEKS AT VARIOUS TEMPERATURES USING MODIFIED BAERMANN SIEVES

AT 23°C

TABLE I

Time taken to recover *Heterorhabditis* and *Steinernema* infective juvenile nematodes from sand[a] after storage for 1-32 weeks at various temperatures using modified Baermann sieves at 23°C

Nematode species/strains	Recovery Time[b] (Days) at 23°C	28°C Time (Weeks) 0	2	4	8	16	32	23°C No. infective juvenile nematodes recovered from sand[a] using a modified Baermann sieve Time (Weeks) 0	2	4	8	16	32
Heterorhabditis sp. D1 Initial no. nematodes per replicate 990±28	1	997±20	28±7	5±6	0	-	-	985±36	63±2	16±6	0	-	-
	2	0	4±3	1±1	-			0	2±1	0	-		
	3	-	0	0	-			-	0	-	-		
	4	-	-	-	-			-	-	-	-		
	5	-	-	-	-			-	-	-	-		
	Total No. Nematodes Recovered	997±20	32±11	6±5	0			985±36	65±1	16±6	0		
H. helvothidis T327 Initial no. nematodes per replicate 1030±28	1	986±20	479±241	238±8	24±3	0	-	1031±65	35±19	11±8	0	-	-
	2	0	19±7	15±4	2±0	-		0	0	0	-		
	3	-	1±1	5±2	2±1			-	-	-	-		
	4	-	0	0	0			-	-	-	-		
	5	-	-	-	-			-	-	-	-		
	Total No. Nematodes Recovered	986±20	498±233	257±3	28±4	0		1031±65	35±19	11±8	0		
Steinernema feltiae Agriotos strain Initial no. nematodes per replicate 1032±83	1	1055±33	88±54	27±26	49±46	2±1	-	1005±16	64±18	4±0	4±1	4±1	0
	2	0	27±13	5±4	4±1	0		0	18±3	1±0	1±1	5±2	-
	3	-	7±7	2±0	2±2	-		-	6±1	1±0	0	0	-
	4	-	5±1	0	0	-		-	2±2	1±0	-	-	-
	5	-	3±3	-	-	-		-	0	0	-	-	-
	Total No. Nematodes Recovered	1055±33	131±78	34±30	54±44	2±1		1005±16	89±20	7±0	5±1	9±1	0
S. glaseri KG strain Initial no. nematodes per replicate 1035±64	1	1016±27	1048±38	950±129	980±259	981±320	488±11	954±41	981±50	963±46	869±121	878±145	315±13
	2	0	0	0	0	0	45±60	0	0	0	0	0	4±2
	3	-	-	-	-	-	3±3	-	-	-	-	-	0
	4	-	-	-	-	-	0	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-
	Total No. Nematodes recovered	1016±27	1048±38	95±129	980±259	981±320	535±53	954±41	981±450	963±46	869±121	878±145	319±11

TABLE I (Continued)

Nematode species/strains	Recovery Time[b] (Days) at 23°C	15°C Time (Weeks) 0	2	4	8	16	32	10°C Time (Weeks) 0	2	4	8	16	32
Heterorhabditis sp. D1 Initial no. nematodes per replicate 990±28	1	987±12	497±33	292±22	79±15	55±14	3±1	1025±20	11±4	0	-	-	-
	2	0	40±30	14±6	0	9±4	2±1	0	12±9	0			
	3	-	2±3	1±1	-	3±2	0	-	14±8	-			
	4	-	0	-	-	0	-	-	7±2	-			
	5	-	-	-	-	-	-	-	4±4	-			
	Total No. Nematodes Recovered	987±12	539±1	306±30	79±15	67±17	5±0	1025±20	47±28	0			
H. heliothidis T327 Initial no. nemtodes per replicate 1030±28	1	1028±14	419±72	281±103	30±10	5±0	2±1	1015±10	502±68	684±52	456±144	113±101	0
	2	0	7±1	1±1	0	1±1	1±1	0	1±1	4±3	6±6	2±2	-
	3	-	0	0	-	0	0	-	0	1±1	2±2	0	
	4	-	-	-	-	-	-	-	-	0	0	-	
	5	-	-	-	-	-	-	-	-	-	-	-	
	Total No. Nematodes Recovered	1028±14	426±71	282±104	30±10	6±1	3±1	1015±10	503±68	689±55	463±136	115±103	0
Steinernema feltiae Agriotos strain Initial no. nematodes per replicate 1032±83	1	1028±74	237±8	105±29	44±26	33±4	27±4	1028±74	780±85	572±62	793±149	542±410	320±150
	2	0	61±19	59±11	12±11	5±2	1±1	0	119±24	131±51	130±47	241±267	123±100
	3	-	11±13	25±1	6±5	0	0	-	26±1	37±12	44±18	9±5	105±63
	4	-	2±1	22±7	2±2	-	-	-	18±9	43±23	25±16	5±2	47±4
	5	-	0	9±1	0	-	-	-	18±22	43±39	11±6	0	0
	Total No. Nematodes Recovered	1028±74	310±25	219±32	63±45	38±7	28±4	1028±74	960±77	825±85	1002±62	797±151	595±118
S. glaseri KG strain Initial no. nematodes per replicate 1035±64	1	1013±15	983±30	1015±91	1036±389	1125±69	899±168	1035±16	990±128	725±161	786±102	804±42	630±55
	2	0	0	0	0	0	7±9	0	0	0	0	7±5	0
	3	-	-	-	-	-	0	-	-	-	-	0	-
	4	-	-	-	-	-	-	-	-	-	-	-	
	5	-	-	-	-	-	-	-	-	-	-	-	
	Total No. Nematodes Recovered	1013±15	983±30	1015±91	1036±389	1125±69	906±159	1035±16	990±128	725±161	786±102	811±37	630±55

[a] Sand moisture content 7% (pF=1.3). [b] Each nematode/temperature/time combination was replicated 4 X.